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# GRAS MONOGRAPH SERIES

# TALL OIL

## (COPIES OF ARTICLES CITED BUT NOT USED IN MONOGRAPH SUMMARY)

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**DEPARTMENT OF HEALTH, EDUCATION AND WELFARE**

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## NEW EXPERIMENTS IN ARTERIOSCLEROSIS

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A. Skeletal muscle suffers greatly in experimental cholesterol arteriosclerosis. There are two types of changes, not necessarily concomitant: (1) hyalinization, calcification, and nuclear proliferation in muscle fibers and (2) subendothelial lipoid cushions in arterioles. Guinea pigs are more gravely affected than rabbits. Denervation prior to cholesterol feeding prevents cholesterol damage. This seems to be a parallel to the findings of Pappenheimer; namely, that denervation prevents muscle damage by avitaminosis E.

B. The effect of stigmasterol on rabbits was studied to determine if it causes changes similar to cholesterol. This was tried on the grounds that stigmasterol is a phytosterol but resembles cholesterol in its formula. Moreover it does not change to any of the vitamins D. The results with feeding of stigmasterol (0.3 Gm. daily for 73 to 116 days) were negative.

C. In addition to rabbits and guinea pigs, golden hamsters also are susceptible to cholesterol arteriosclerosis. In contrast, rats are refractory. This may be explained by the fact that the latter are omnivores and readily dispose of the cholesterol. Recently, gophers were fed, for two to seven months, a milk-egg-yolk diet. None of these rodent herbivores showed cholesterol damage except cholesterol gallstones. Some foci of calcification were present, caused, perhaps, by the increased intake of vitamin D.

## THE CLINICAL DIAGNOSIS OF ARTERIOSCLEROSIS WITH PARTICULAR REFERENCE TO THE USE OF THE ROENTGEN RAY

FREDERIC D. ZEMAN AND MAX SCHENCK, NEW YORK, N. Y.

No abstract.

Ind. J. Genet. Plant Breeding. 29: 276-279, 1969

# EFFECTS OF FATTY ACIDS ON HUSKLESS BARLEY

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(Accepted: 7-ii-1969)

In recent years many organic and inorganic compounds have been used as mutagens. It has been shown that a number of essential and vegetable oils also produce chromosomal aberrations as well as mutations (D'Amato and Avenzi, 1949; Swaminathan and Natarajan, 1956, 1959). As far as known to the authors use of fatty acids as mutagens has not been reported so far.

Investigation presented here form a part of studies initiated in this department to study the mutagenic effects of various fatty acids on crop plants.

## Materials and Methods

$M_1$ and $M_2$ generations of huskless barley seeds which had been soaked in oleic acid and ricinoleic acid for 4 hours and 12 hours under dry and wet (pre-soaked in water) conditions were studied. The unsaturated fatty acids were obtained locally.

Seeds of $M_1$ and $M_2$ generations were sown in complete randomized design with three replications. Observations were taken on plant survival, plant height number of days taken to ear emergence, length of ear, number of nodes per plant, number of tillers/plant, weight of 1,000 grains, yield per plant, pollen fertility and mitotic chromosome aberrations.

## Results and Discussion

From the data presented in Tables 1 and 2 it will be observed that fatty acid treatments markedly reduced the plant survival and plant height, but increased the number of days to ear emergence both in $M_1$ and $M_2$ generations. The reduction was more evident in wet treatments as compared to the dry treatments. In both $M_1$ and $M_2$ generations, as the duration of oleic acid treatment increased, there was progresive decrease in plant survival and plant height. Swaminathan and Natarajan (1956,1959) also reported reduction in germination and pollen fertility in wheat which had been treated with different oils.

Variations in other morphological characters like length of ear, number of nodes per plant, number of tillers per plant, weight of 1,000 grains and yield per plant were not significant either in $M_1$ and $M_2$ generations.





Table 1

*Morphological and cytological observations in $M_1$ generations of fatty acid treated barley*

| Treatments | Duration | Mean values for | | | | Percentage of mitotically aberrent cells |
|---|---|---|---|---|---|---|
| | | Plant survival % | Plant height (cm.) | Pollen fertility % | Days to ear emergence | |
| Oleic acid (dry) | 4 hrs. | 82·3 | 61·4 | 98·7 | 41·2 | 3·8 |
| " " " | 12 hrs. | 76·7 | 55·6 | 98·1 | 40·66 | 7·0 |
| " " (wet) | 4 hrs. | 48·6 | 46·5 | 96·9 | 54·6 | 9·67 |
| " " " | 12 hrs. | 44·2 | 50·1 | 98·8 | 58·3 | 12·01 |
| Ricinoleic acid (dry) | 4 hrs. | 88·5 | 62·9 | 96·2 | 40·6 | 3·07 |
| " " " | 12 hrs. | 76·8 | 59·8 | 98·4 | 44·0 | 7·06 |
| " " (wet | 4 hrs. | 46·2 | 48·7 | 98·8 | 42·6 | 7·21 |
| " " " | 12 hrs. | 62·7 | 50·5 | 98·4 | 46·6 | 12·04 |
| Control | | 98·2 | 60·6 | 99·1 | 40·0 | — |

TABLE 2

*Morphological and cytological observations in $M_2$ generation of fatty acid treated barley*

| Treatments | Duration | Mean values* for | | | | No. of chlorophyll mutations per 100 $M_2$ seedlings | Chromosomal aberrations | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Plant survival % | Plant height (cm) | Pollen fertility % | Days to ear emergence | | No. of cells scored | Bridges | Fragments | No. of cells affected | % of aberrant cells |
| Oleic acid (dry) | 4 hrs. | 73·7 | 47·2 | 79·4 | 69·3 | 1·6 | 134 | 7 | 25 | 29 | 21·6 |
| " " " | 12 hrs. | 59·8 | 52·4 | 87·9 | 66·3 | 2·4 | 191 | 15 | 38 | 49 | 25·7 |
| " " (wet) | 4 hrs. | 27·7 | 46·3 | 84·8 | 90·0 | 3·6 | 165 | 19 | 51 | 64 | 38·8 |
| " " " | 12 hrs. | 18·7 | 47·6 | 85·4 | 88·3 | 1·4 | 171 | 23 | 50 | 68 | 39·8 |
| Ricinoleic acid (dry) | 4 hrs. | 86·2 | 46·8 | 77·9 | 71·0 | 1·8 | 161 | 10 | 29 | 38 | 23·6 |
| " " " | 12 hrs. | 66·5 | 49·5 | 78·8 | 67·3 | 4·7 | 172 | 19 | 30 | 49 | 28·5 |
| " " (wet) | 4 hrs. | 25·5 | 48·1 | 90·3 | 73·0 | 1·3 | 158 | 9 | 24 | 33 | 20·9 |
| " " " | 12 hrs. | 42·5 | 45·7 | 87·1 | 75·7 | 1·4 | 167 | 15 | 45 | 56 | 33·5 |

*Mean of 15 observations per replications.



In $M_1$ generation, there was no effect on pollen fertility, but $M_2$ plants showed significant reduction in pollen fertility. This reduction in fertility could be partially due to effect of environment and of mitotic disturbances. Chromosomal aberrations, chiefly high number of bridges and fragments, were scored; percentage of aberrant cells increased in $M_2$ plants as compared to $M_1$ generation. The variations in plant survival, plant height and days to ear emergence between $M_1$ and $M_2$ generations can be explained as an environmental effect.

A narrow spectrum and a low frequency of chlorophyll mutations were recorded only in $M_2$ generation. Low frequencies (Table 2) of *virdis* type mutants were observed but *albino* types were not seen. Similar results were also reported by Ehrenberg, Gustafsson and Lundquist (1961). They concluded that treatments with chemical mutagens produced relatively low frequency of *albino* mutants as compared to *virdis* and other types.

Induced viable mutations were not detected in $M_1$ and $M_2$ generations, although ear emergence was delayed in treatments and a few plants with late maturity were isolated.

Chromosomal abnormalities like fragmentation, stickiness and chromosome bridges were observed in root tips in all treatments. Percentage of aberrant cells were noticed more in wet than in dry treatments of oleic acid; however, there was little variation in case of ricinoleic acid treatment. Swaminathan and Natarajan (1956, 1959) also observed chromosome and chromatid breaks, binucleate cells and abnormalities in root tips of *Triticum* treated with different oils. They suggested that unsaturated fatty acids may form the mutagenic fraction of vegetable oils. However, results presented here revealed that unsaturated fatty acids, though they produce chromosomal aberrations, are not efficient mutagens.

## SUMMARY

Biological effects of fatty acid treatment on huskless barley were observed. Chlorophyll mutants were detected in low frequency, whereas viable mutants were absent. Significant variations were observed only in plant height and ear emergence. Chromosomal aberrations were seen in all the treatments.

## ACKNOWLEDGEMENTS

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Swaminathan, M. S. and Natarajan, A. T. (1956). Chromosome breakage induced by vegetable oils and edible fats. *Curr. Sci.*, **25**: 382–84.

021

# CANADA

# THE PATENT OFFICE

PATENT No. 522,998 ISSUED MAR. 20, 1956

## Manufacture of Cellulose

Frank R. Charles and Julian A. Dixon, Hawkesbury, Ontario, Canada, assignors to Canadian International Paper Company, Montreal, Quebec, Canada

Application August 31, 1951, Serial No. 819,954
In the United States August 20, 1951
8 Claims — No drawing

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This invention relates to new and useful improvements in the manufacture of cellulose, and especially cellulose suitable for conversion into viscose rayon, cellophane, sausage casings, cellulose derivatives such as nitrocellulose, cellulose acetate and the like, and other cellulosic products. Particularly, this invention relates to the manufacture of cellulose from wood or woody materials and has many advantages over previous wood pulps when employed to make viscose rayon. The process disclosed herein is also applicable to the processing of cotton which is to be used for similar purposes.

In the manufacture of the highest quality wood cellulose, wood is debarked and chipped, and the wood is treated at elevated temperatures and pressures with solutions of chemicals. Such chemicals are either bisulphites, usually of calcium, magnesium or ammonium and containing an excess of free $SO_2$ in solution, or are alkaline solutions of sodium hydroxide, sodium sulphide, sodium carbonate, or mixtures of these. At the conclusion of this treatment the softened chips are disintegrated into wood pulp. Such wood pulp contains small but significant proportions of lignin, tannins and other coloring matter, and organic solvent-extractable materials such as fats, fatty acids, resinous acids, sterols and hydrocarbons. The organic solvent-extractable materials are collectively termed "resin." The wood pulp produced as described above, which is mainly cellulose, is purified and bleached by one of several known processes for reaching commercially acceptable levels of purity of the cellulose. As the art of purifying cellulose has improved, resulting in nearly pure cellulose with a very small content of non-cellulosic constituents, many advantages have accrued, particularly in the properties of the rayon or other derivatives manufactured from such purified cellulose. Examples of such properties are color and strength. At the same time certain difficulties have arisen in the processing of such cellulose. For example, the lowering of the resin content of rayon-grade cellulose has given rise to difficult filtration of the viscose made from it.

Simultaneously, conditions in the rayon-making industry have changed, particularly in the direction of higher speeds in various processing steps, requiring cellulose which will process more rapidly.

We have discovered that these difficulties can be

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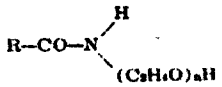
overcome and a superior pulp for processing can be made by adding to the cellulose at a late stage in its manufacture one or more ethenoxy N-monoethanolamides of refined tall oil. Such products have the generic formula:



Where R is an alkyl and/or aryl radical derived from refined tall oil and n is an integer between 4 and 50.

Black liquor soap, as formed in the manufacture of wood pulp by the sulphate or Kraft process, is removed from the pulp mill recovery system and converted to crude tall oil. The crude tall oil may be refined by steam distillation, solvent extraction, or other methods to provide the intermediate material from which the condensation products used in accordance with the teachings of this invention may be formed. The condensation products to be used in accordance with this invention are prepared by first reacting a refined tall oil with monoethanolamine in substantially equimolecular quantities at temperatures sufficiently high to produce a monoethanolamide of the refined tall oil, followed by condensation of the amide with from 4 to 50 mols of ethylene oxide per mol of the amide to form an ethenoxy N-monoethanolamide of refined tall oil. One such product which has been found satisfactory in the practice of the present invention may be formed from a refined tall oil known as "Acintol D", as presently manufactured by the Arizona Chemical Company and consists approximately of 33% abietic acid, 5% unsaponifiable materials and the balance of fatty acids, generally unsaturated of 18 carbon atoms. Other refined tall oils will be found equally suitable for the purposes of this invention, although the above mentioned material has proven to be particularly satisfactory.

As far as we know condensation products made from ethylene oxide and the amides of tall oil acids are new, and this invention is believed to represent the first use of such products for improving the manufacture of cellulose derivatives.

These chemicals show remarkable stability in the viscose process being stable under both the alkaline conditions of viscose preparation and the acid conditions of the spinning bath.

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Benefits will be conferred by 4 to 50 ethylene oxide units in the ethenoxy portion of these amide condensation products, but best results are obtained with 10 to 30 ethylene oxide units.

We have found that small quantities of these products added to the cellulose confer very marked benefits. Among advantages found in the manufacture of viscose rayon is improved shredding of the alkali-cellulose, less power being required to shred the sheets to the same degree of comminution. The xanthation reaction is more uniform, with an apparent possibility of economy in carbon disulphide requirements, and there is a substantial reduction in the unreacted fibre content of the viscose and therefore much improved filtration of the viscose. Spinning is improved, spinneret incrustation reduced and rayon strength improved The fatigue of rayon tire cord is improved.

Cellulose manufactured according to this invention also has special advantage in the manufacture of dull yarn made with suspensions of pigments and oils in the viscose since the suspensions are much more fine grained and stable and the spinning proceeds with many fewer interruptions due to plugging or constriction of spinneret holes.

These products can be added easily to the cellulose. One way is to apply them in dilute aqueous solution to the cellulose at an early stage in the drying of the pulp sheet, in such a way that the added water is evaporated during the further drying of the sheet. An aqueous solution may be sprayed on the cellulose sheet or it may be applied by means of rolls. Narrow stripes may be applied by means of discs as it has been found that sufficient dispersion occurs in the shredding stage.

In accomplishing the objects of this invention it is not necessary to use more than 0·2% on the weight of cellulose and usually 0·01% to 0·1% is sufficient.

The following two examples will illustrate preferred embodiments of this invention, but the invention must not be construed as limited to these examples.

*Example I*

A 10% aqueous solution of a product obtained by reacting a refined tall oil ("Acintol D") acid amide with 25 mols of ethylene oxide was sprayed continuously across a sheet of cellulose passing through a pulp drying machine at such a rate that 0·1% was added based on weight of bone dry cellulose. The sheet was dried to the normal moisture content (about 7%). The cellulose treated in this manner exhibited the following desired improvements, namely improved shredding of the alkali-cellulose, more uniform xanthation reaction, substantial reduction in the unreacted fibre content of the viscose and therefore improved filtration of the viscose, improved spinning, reduced spinneret incrustation, improved rayon strength and lesser fatigue of rayon tire cord.

*Example II*

A 50% solution of a product obtained by reacting a refined tall oil ("Acintol D") acid amide with 20 mols of ethylene oxide was applied to high quality refined sulphite pulp by means of $\frac{1}{16}$" face discs dipping into the liquid heated to 65°C. The dried cellulose sheet was passed over the discs causing them to rotate and imprint the compound on the cellulose sheet. The spacing of the discs was such that 0·1% of the compound

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was added, based on the weight of cellulose.

The cellulose treated in this fashion exhibited the following improvements in processing into 1650 denier 720 filament high strength viscose rayon tire yarn, spinning at 100 metres per minute:

| | Treated Wood Pulp | Untreated Wood Pulp |
|---|---|---|
| Shredding of alkali-cellulose | | |
| % retained on ¼" mesh screen after 1½ hours shredding | 4·0 | 8·5 |
| Nature of Alkali-Cellulose Crumb | Soft, fluffy and reactive | Rather hard in feel |
| Viscose Filtration | | |
| Weight in grams filtered through a ½" diameter disc of Canton flannel before filter plugged | 126 | 100 |
| Rayon Strength | | |
| Grams per denier, bone-dry | 4·00 | 3·94 |
| % Elongation, bone-dry | 16·1 | 15·9 |
| Grams per denier, conditioned | 3·55 | 3·35 |
| % Elongation, conditioned | 20·9 | 19·9 |
| Grams per denier, wet | 2·16 | 2·07 |
| % Elongation, wet | 24·4 | 24·4 |
| Tire Cord (1650 denier 2 ply cord) | | |
| Grams per denier, bone-dry | 3·33 | 3·23 |
| % Elongation, bone-dry | 11·0 | 10·9 |
| Grams per denier, conditioned | 2·86 | 2·75 |
| % Elongation, conditioned | 14·3 | 14·7 |
| Fatigue, D.B.—minutes | 40·8 | 34·2 |

Thus it will be seen that marked improvements are effected in alkali-cellulose shredding, viscose filtration and tire cord fatigue, while noticeable improvements are seen in almost all of the remaining properties tested

The presence of these products also reduces the degradation of bleached viscose rayon that occurs on prolonged heating. The following results were obtained after bone-drying bleached rayon in an oven at 105° for 16 hours and then reconditioning for 24 hours before testing:

| | Treated Wood Pulp | Untreated Wood Pulp |
|---|---|---|
| Grams per denier | 3·53 | 3·04 |
| % Elongation | 21·4 | 18·4 |

Many variations of this process will suggest themselves to those skilled in the art, but such variations are considered as falling within the scope of this invention, provided the products employed are within the class described.

The products may be added at any stage up to and including the viscose itself, but with diminishing benefits. This will be obvious from our disclosure since addition to the viscose, for example, cannot give improved shredding or any of the other benefits derived in the manufacturing of the viscose However, addition even to the viscose itself confers the substantial advantages of improved dispersion and stability of pigments and oils, better spinning with fewer breaks, less spinneret incrustation, more first grade yarn, higher rayon strength, and resistance of bleached rayon to degradation by heating.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. In the manufacture of cellulose derivatives from sheeted purified chemical pulp by the viscose process, the step of incorporating into said process at a stage subsequent to the formation of the pulp sheets from 0·01% to 0·2% of ethenoxy N-monoethanolamides of refined tall oil wherein the num-

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ber of ethylene oxide units is from 4 to 50 per amide molecule.

2. A method for improving the processibility of purified chemical pulp into cellulose derivatives which comprises adding to said purified chemical pulp at least one of the ethenoxy N-monoethanolamides of refined tall oil, wherein the number of ethylene oxide units is from 4 to 50 per amide molecule, said amides being added in an amount from 0·01% to 0·2% based on the bone-dry weight of said pulp.

3. A method for improving the processibility of purified chemical pulp into cellulose derivatives which comprises adding to said purified chemical pulp at least one of the ethenoxy N-monoethanolamides of refined tall oil in an amount from 0·01% to 0·2% based on the bone-dry weight of said pulp, said refined tall oil before condensation consisting approximately of 33% abietic acid, 5% unsaponifiable materials and the balance of fatty acids, generally unsaturated of 18 carbon atoms.

4. A method for improving the processibility of purified chemical pulp into cellulose derivatives which comprises adding to said purified chemical pulp at least one of the ethenoxy N-monoethanolamides of refined tall oil, wherein the number of ethylene oxide units is from 4 to 50 per amide molecule, said tall oil before condensation consisting approximately of 33% abietic acid, 5% unsaponifiable materials and the balance of fatty acids, generally unsaturated of 18 carbon atoms.

5. In the manufacture of regenerated cellulose products by the viscose process, the step of incorporating into viscose ethenoxy N-monoethanolamides of refined tall oil wherein the number of

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ethylene oxide units is from 4 to 50 per amide molecule, said amides being added in an amount from 0·01% to 0·2% based on the weight of the cellulose content of the viscose.

6. In the manufacture of regenerated cellulose products by the viscose process, the step of incorporating into viscose ethenoxy N-monoethanolamides of refined tall oil wherein the number of ethylene oxide units is from 4 to 50 per amide molecule, said refined tall oil before condensation consisting approximately of 33% abietic acid, 5% unsaponifiable materials and the balance of fatty acids, generally unsaturated of 18 carbon atoms, said amides being added in an amount from 0·01% to 0·2% based on the weight of the cellulose content of the viscose.

7. A new article of manufacture consisting of a purified chemical cellulose pulp product having incorporated therein ethenoxy N-monoethanolamides of refined tall oil wherein the number of ethylene oxide units is from 4 to 50 per amide molecule, said refined tall oil before condensation consisting approximately of 33% abietic acid, 5% unsaponifiable materials and the balance of fatty acids, generally unsaturated of 18 carbon atoms, said amides being present in an amount from 0·01% to 0·2% based on the bone-dry weight of said pulp.

8. A new article of manufacture consisting of a purified chemical cellulose pulp product having incorporated therein from 0·01% to 0·2% of the ethenoxy N-monoethanolamides of refined tall oil wherein there are from 4 to 50 ethylene oxide units per amide molecule.

EDMOND CLOUTIER, C.M.G., O.A., D.S.P., Queen's Printer and Controller of Stationery, Ottawa, 1956

SECTION OF THE

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122 E. 42nd ST., NEW YORK 17, N. Y. Edited By R. G. MACDONALD, Secretary

# Physiological Effects of Sulphate Pulp Mill Wastes on Shellfish*

By Walter A. Chipman, Jr.[1]

**Abstract**

*The addition of sulphate pulp-mill wastes to sea water reduced the hours that oysters remained open and decreased the rate of pumping of water through the gills. The depression of the rate of filtration was found to be proportional to the concentration of effluent.*

*Strong kraft soap, weak soap, and black liquor were found to contain this physiologically active material in great amount. It tends to concentrate in the soap portion of the mill products. Oxidation of the weak black liquor makes the material ineffective.*

*The toxic substance can be precipitated from mill liquors by salt. Its presence in the soap is due to adsorption by the latter of material "salted out" of the black liquor by the treatment with salt.*

The toxic action of sulphate pulp-mill wastes on aquatic animals has been observed and reported many times. These wastes, when present in sufficient concentration, were found to be injurious to fishes as early as 1908 by Ahlin *(1)*. Other European workers, such as Ebeling *(2, 3, 4)*, Vallin *(5)*, Hagman *(6)*, Järnefelt *(7)*, Bergström and Cederquist *(8)*, Vestergren *(9)*, and Erdtman *(10)* have pointed out the toxicity to fresh-water fish of the resin acids present in the sulphate mill effluents. In the United States toxicity studies have been reported for fresh-water fishes by Cole *(11)*, Cole and Warwick *(12)*, Extrom and Farner *(13)*, and others. No work prior to that reported in this paper has been done on the effect of sulphate mill wastes on shellfish. The experimental studies of the physiological action of these pollutants on oysters was carried on in connection with an investigation of a marked decline in the oyster production of the York River, Virginia, which resulted from a failure of the oysters to grow and develop a good quality for market. The complete report of this investigation recently has been published *(14)*.

Although the emphasis on abatement of pollution is rightfully placed on conditions affecting fresh-water streams, more and more attention is being directed to studies of pollution of coastal waters since the number of industrial plants along the coast is being increased. It is in the brackish waters of bays and estuaries that many of the seafoods are produced and pollution of such areas is of importance. This is particularly true of shellfish.

The oyster, being sedentary, is unable to avoid unfavorable environments excepting by closing its shell and remaining closed. If this condition persists for some time, the animal must perish. Any material of the surrounding water that causes the oyster to remain closed will prove injurious. Other substances not causing this response may still damage the organism through interference with its inner mechanisms concerned with feeding and respiration.

Undisturbed on the bottom, the oyster relaxes the adductor muscle and allows the shells to open from the elasticity of the hinge. Sea water is drawn through the gills by the action of multitudes of cilia, microscopic hair-like processes which completely cover the gills. The water is forced through small pores into the water tubes within the gills. Here the oyster blood is oxygenated by close contact with the water within the tubes. This water then passes to a chamber above the gills and is forced out of the shells in a rather rapidly moving stream. As particles of microscopic plants and animals are brought to the gills with the water, they are collected on the gill surface and become embedded in a slime which covers the soft parts of the animal, and, by the action of the minute cilia, are carried across the surface of the gill to and into the mouth. The contractions of the adductor muscle and the mantle, a covering of the body of the animal lining the shell, control the flow of water by regulating the opening and closing of the mantle chamber to provide for a greater or lesser access of the outside water to the gills. Disruption of the pattern of ciliary motion also will stop the flow.

The experimental work herein reported consisted of observations of the effect of pulp-mill effluent on the time oysters remained open, on the pumping activity of the gills of oysters, and on the rate of water filtration of the entire oyster. A few studies were made of the physiological action of kraft soaps and black liquor. Bioassays

TABLE I.—MEAN VALUES OF HOURS OPEN IN VARIOUS CONCENTRATIONS OF PULP-MILL EFFLUENT

| Concentration in Parts per 10 Liters | Mean Value of Hours Open | Standard Deviation | Standard Error |
|---|---|---|---|
| 1,000 | 5 | ... | ... |
| 100 | 8.5 | ... | ... |
| 50 | 12.8 | 2.40 | ± 0.85 |
| 25 | 13.7 | 2.52 | ± 0.89 |
| 10 | 13.5 | 3.89 | ± 1.17 |
| 5 | 17.9 | 1.29 | ± 0.65 |
| 2.5 | 20.5 | 3.15 | ± 1.57 |
| 2.0 | 19.2 | 1.63 | ± 0.67 |
| 1.0 | 20.9 | 2.06 | ± 1.03 |
| 0 | 21.3 | 1.29 | ± 0.26 |

* Presented at the Annual Meeting of the Technical Association of the Pulp and Paper Industry, Hotel Commodore, New York, N. Y., Feb. 23-26, 1948.

[1] Aquatic Biologist, U. S. Fish and Wildlife Service, College Park, Md.

were completed on various fractions of these materials in attempts to isolate the physiologically active substance.

The effluent samples tested, collected from the sewer of the mill at different times, were composites of like unit volumes taken every 15 minutes for a period of 5 hours. Since the waste flowing in the sewer was quite varied in character even these composites showed considerable differences in their specific gravity and in their components. For this reason the dilution used with one particular effluent may not truly correspond in strength with a similar dilution of another, and variations in physiological effectiveness may be expected. Similar difficulty in obtaining effluent samples of comparable composition were reported by Extrom and Farner *(13)*.

### Effect of Pulp-Mill Effluent on Time That Oysters Remain Open

The laboratory experiments of the effect of pulp-mill effluent on the time that oysters remain open were conducted on oysters placed in chambers receiving running sea water or sea water to which known amounts of sulphate mill effluent were being added. By means of a light thread connecting one of the shells of the oyster with one end of a pivoted lever, a tracing of the movement of the shell was obtained on a smoked paper fastened to a revolving kymograph drum. The experiments were carried on for periods from 6 to 30 days using concentrations of effluent in sea water from 1:10 to 1:10,000. Shell movement records were obtained for 49 oysters treated with the effluent and compared with those of 25 oysters in sea water during the same time.

The results varied in the experiments performed with different samples of effluent. However, the number of hours that the oyster remained open was reduced when effluent was present in the sea water in concentrations stronger than 1:10,000. The time the oysters remained open decreased as the concentration was increased. This relationship is clearly seen in Table I which shows the mean values of hours open in various concentrations of the effluent with their standard deviation and standard errors.

A marked physiological response was shown in the type of muscular activity of the oysters subjected to concentrations of effluent 1:1,000 or stronger. Rapid and frequent shell closures gave evidence of an irritating effect immediately after the material was added. These periods of activity were followed by long periods when the oysters remained tightly closed. In very strong concentrations the ability to hold the shells closed was lost. Attempts by the oysters to close their shells after this became less frequent and the strength of the adductor muscle diminished. Long periods when the shells remained open, with only feeble attempts to close at infrequent intervals, were characteristic of the time just preceding death of the oysters.

### Effect of Effluent on Rate of Water Movement by Action of Gill Mechanisms

Tests were made of the effect of various concentrations of effluent on the rate of water filtration by the activity of the gill mechanisms. After prying the shells slightly apart, a glass rod was inserted to prevent the oyster from closing. A small rubber tube was inserted into the chamber from which the water leaves the oyster and the remaining space on the excurrent side was packed with wet cotton. Water entering the oyster on the incurrent side would then leave the oyster through the rubber tube, which in turn was connected to a calibrated glass tube. By adding a small amount of carmine suspension to the water flowing in this tube, it was possible to time between marks the passage of the tip of the red cone thus formed. In other tests the tube was connected to a small chamber from which the incoming water would drop onto an electric drop-counter and the number of drops per minute recorded. These methods have been fully described by Galtsoff *(15, 16)*. Such arrangements would rule out the activity of the shell and mantle in controlling the rate of flow and limit it to that of the gill epithelium.

In a number of experiments the oysters were subjected to increasing concentrations of the pulp-mill effluent in sea water. In other tests the oysters were kept in one concentration of effluent for several hours. Readings generally were made after half hour exposure to each concentration or at half hour intervals in a fixed concentration of pollutant.

The material physiologically active in altering the rate of flow was present in varying amounts in the different samples. In certain ones concentrations of 1:2,000 and 1:4,000 were effective. In others, the only changes were those brought about by exposure to strengths of 1:100 or 1:2000. The reduction of the rate of water filtration resulting from one of the samples is shown in Table II. The percentage of depression in the rate of pumping of water through the action of the gill epithelium was inversely proportional to the concentration of the effluent.

It was observed also that the effects were temporary and that the rate of pumping returned to its original value once the pollutant was removed. There were no irreparable changes in the gill epithelium after several hours of exposure to the concentrations used in the experiments. It can be inferred that there was no cumulative effect on the ciliary mechanism under conditions of the tests.

TABLE II.—DEPRESSION OF THE RATE OF FILTRATION CAUSED BY PULP-MILL EFFLUENT (SAMPLE 24)

[Specific gravity 1.0043. Carmine-cone method. Figures express means of percentages of normal rate. Each oyster was exposed to one concentration of the effluent for 1½ hours. Three sets of readings weres made at half-hour intervals.]

| Concentration, parts per liter | Number of Oysters | Percentage of Normal Rate | Standard Deviation | Percentage of Depression |
|---|---|---|---|---|
| 0 ..... | 21 | 100 | ..... | 0 |
| 0.25 ..... | 6 | 99.2 | 4.37 ± 0.87 | 0.8 |
| 0.5 ..... | 18 | 89.2 | 8.21 ± 1.90 | 10.8 |
| 1.0 ..... | 27 | 77.8 | 5.83 ± 1.12 | 22.2 |
| 2.0 ..... | 15 | 36.5 | 5.65 ± 1.46 | 63.5 |

### Effect of Pulp-Mill Effluents on Rate of Pumping of Water by Intact Oysters

Measurements were made of the amount of water pumped by oysters exposed to pulp-mill effluent and to pure sea water under the combined action of all the mechanisms which control the rate of water filtration; namely, that of the ciliated epithelium of the gills and their ostia, the mantle, and the adductor muscle.

The so-called Rubber Apron method was used which involves the technique developed by Nelson *(17)*. In this the oyster is so wrapped in a piece of rubber dam that a cone is formed leading from the excurrent side. All the water entering the oyster must leave through this cone. By mounting the oysters in a constant level tank the flow of the water from the oyster is collected in a chamber which discharges when filled to a certain level, the volume at which it empties being known. A record is maintained automatically of the shell movement and the amount of water pumped by the oyster.

The amount filtered by individual oysters varies considerably according to size. For this reason a basic rate of pumping was determined for each experimental oyster from records of its activity in plain sea water. The rate of pumping during exposure to various concentrations of effluent was then measured and compared to this basic rate. For each the mean rate was determined, counting only the hours when the shell was open. The fact that

the shells are open does not necessarily mean the oyster is feeding, for many observations had shown that the oysters could remain open and still not pump water.

The results (Table III) show that concentrations of mill effluent of 1:200 and 1:400 gave immediate and marked effects, depressing the water output to a small fraction of its rate observed before treatment. In concentrations of 1:770 and 1:1,000 the effects were less pronounced. Some oysters appeared unaffected and others showed a marked drop in the rate of flow.

For a picture of the combined effects we may consider an analysis of the data of one test, which was typical of the others, and is shown in Table IV. Both the rate of pumping when open and the hours open were reduced. As a result the total daily amount of water filtered was decreased by the action of the effluent in the sea water with a total for the 12½ days of 760.3 liters for the test oyster and 2259.5 liters pumped by the control, a difference of approximately 1,500 liters.

## Effects of Mill Products on Pumping of Water by Gills of Oysters

Physiological tests were made of the various mill products which were found in varying amount and at different times in the effluents. These tests measured the effectiveness of a 30-minute exposure time on reducing the filtering activity of the oyster gills. It was found that strong kraft soap in dilutions of 1:200,000 was very effective. Weak soap and black liquor also caused immediate and marked reduction in relatively dilute concentrations.

Storage of effluent samples open to the air only slightly reduced their effectiveness. Drying of a sample of effluent reduced its ability to alter the rate of water pumping by the gills of oysters, but did not completely destroy it. Ashing of the dried residue caused a complete loss of physiological activity of the material under the conditions of the experiment. A sample of black liquor stored under hydrogen for a period of 14 months was found to retain its potency. Other samples similarly stored only slightly lost their effectiveness in reducing the rate of water filtration of oysters under the experimental conditions.

One sample of black liquor was placed in a flask and the air above displaced with pure oxygen. The flask and its contents, connected to a reservoir containing a known volume of oxygen, was shaken for 90 hours. It was found that the black liquor thus oxidized had lost its ability to reduce the rate of pumping of water by the oysters.

Samples of weak black liquor and kraft soaps were treated in various ways in attempts to isolate in some particular fraction the material responsible for the physiological activity. This involved salt precipitation in the presence of ether and extraction with ethanol and methanol. Bioassays were made of the materials obtained at various stages in the scheme of fractionation. These consisted of examination of the rate of water filtration of oysters exposed to increasing concentrations of the material under observation using the carmine cone technique.

It was demonstrated that the toxic material affecting oysters tends to concentrate in the soap portion of the mill products. This is in agreement with the conclusions of a number of European investigators who ascribe the toxicity to fishes as due to the resin acids and their sodium and calcium salts.

The experimental studies showed that the toxic material could be separated from the soap and from weak black liquor by fractionation. Treatment of mill liquors with salt caused a "salting out" of the effective substance but repeated treatment of the weak black liquor was necessary to accomplish this completely. The sulphate soaps precipitated from the mill liquor by the action of the salt in the presence of ether did not contain the toxic material but the ether extracts were effective.

The evidence indicates that these soaps, when precipitated from the mill liquors, adsorb the toxic substance of these liquors and that this effective material is a constituent of the unsaponifiable portion.

The exact nature of the toxic material was not determined in the work reported in this paper. Erdtman has definitely shown that the toxic substance obtained from the European pine, *Pinus sylvestris*, is a dioxystilbene present in the heartwood of the tree.

TABLE III.—EFFECTS OF PULP-MILL EFFLUENT ON RATE OF PUMPING OF OYSTERS (RUBBER-APRON METHOD)

[Rate of pumping of test oysters is expressed in percentage of basic rate determined from records obtained during the number of days these oysters were kept in sea water before pollutant was added. Basic rate for control has been determined for the same periods of time.]

| Concentration | Sample No. | Effluent Specific Gravity at 17.5° C. | Oyster No. | Days in Sea Water | Days in Effluent | Rate of Pumping in Per Cent of Original Rate | |
|---|---|---|---|---|---|---|---|
| | | | | | | Control | Test |
| 1:200 | 3 | 1.0013 | 1 | 3 | 6 | 119.1 | 4.4 |
| 1:200 | 3 | 1.0013 | 3 | 3 | 8 | 107.5 | 9.4 |
| 1:400 | 6 | 1.0012 | 9 | 3 | 1 | 97.5 | 46.4 |
| 1:400 | 6 | 1.0012 | 8 | 3 | 4 | 93.9 | 25.6 |
| 1:770 | 6 | 1.0012 | 12 | 3 | 5 | 105.1 | 16.2 |
| 1:770 | 6 | 1.0012 | 11 | 2 | 6 | 92.4 | 84.2 |
| 1:1,000 | 5 | 1.0013 | 3 | 2 | 4 | 99.5 | 114.6 |
| 1:1,000 | 5 | 1.0013 | 2 | 1 | 6 | 111.6 | 125.7 |
| 1:1,000 | 6 | 1.0012 | 10 | 6 | 5 | 125.1 | 102.5 |
| 1:1,000 | 24 | 1.0043 | 204 | 4 | 1 | 128.2 | 22.1 |
| 1:1,000 | 24 | 1.0043 | 205 | 2 | 2 | 110.4 | 70.7 |
| 1:1,000 | 50 | 1.0022 | 102 | 2 | 3 | 156.0 | 39.9 |
| 1:1,000 | 51 | 1.0014 | 104 | 2 | 13 | 161.6 | 46.6 |

From these tests it can be seen that it is possible to render the active material of the effluent physiologically inactive by oxidation but that it is not readily oxidized.

TABLE IV.—EFFECT OF PULP-MILL EFFLUENT (SAMPLE 51) DILUTED 1:1000 ON THE RATE OF PUMPING PER HOUR AND THE TOTAL AMOUNT OF WATER FILTERED BY THE OYSTERS DURING EACH DAY

[Difference between control and experimental = 1,499.2 liters.]

| Day of Observation | Experimental | | | Control | | |
|---|---|---|---|---|---|---|
| | Time Open, hr. | Rate of Pumping, l./hr. | Total Water Filtered, l. | Time Open, hr. | Rate of Pumping, l./hr. | Total Water Filtered, l. |
| 1 | 24.0 | 7.4 | 177.6 | 24.0 | 4.8 | 115.2 |
| 2* | *12.0 | *9.8 | *235.2 | *11.1 | *6.0 | *133.2 |
| 2** | **6.1 | **2.2 | **26.8 | **11.2 | **6.0 | **134.4 |
| 3 | 11.1 | 1.4 | 15.5 | 21.2 | 7.9 | 167.5 |
| 4 | 16.8 | 1.6 | 26.9 | 21.6 | 10.1 | 218.2 |
| 5 | 14.5 | 3.1 | 45.0 | 21.9 | 9.9 | 216.8 |
| 6 | 16.2 | 2.1 | 34.0 | 20.2 | 10.4 | 210.1 |
| 7 | 16.4 | 3.1 | 50.8 | 20.9 | 8.1 | 169.3 |
| 8 | 15.9 | 2.8 | 44.5 | 16.1 | 6.2 | 99.8 |
| 9 | 12.9 | 3.7 | 47.7 | 15.8 | 7.3 | 115.3 |
| 10 | 16.2 | 4.1 | 66.4 | 22.1 | 7.7 | 170.2 |
| 11 | 16.0 | 7.0 | 112.0 | 23.2 | 8.2 | 190.2 |
| 12 | 14.3 | 6.7 | 95.8 | 21.2 | 9.4 | 199.3 |
| 13 | 15.8 | 6.9 | 109.0 | 21.0 | 8.7 | 182.7 |
| 14 | 12.1 | 7.4 | 99.3 | 23.2 | 10.9 | 252.9 |
| Total for 12½ days of test | ... | ... | 760.3 | ... | ... | 2,259.5 |

* Observations made 12 hours before addition of test material to experimental chambers.

** Observations made 12 hours after adding of test material to experimental chambers.

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# A Study of the Influence of Storing Wood on the Yield and Quality of Tall Oil

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**Abstract**

In 12 weeks of storage time pine roundwood lost approximately 11% in tall oil yield, while for the same length of time purchased slabwood chips (pine) lost 64%. Most of the loss in yield occurred within six weeks. The purchased chips lost more tall oil yield in one week than the roundwood in 12 weeks.

The loss in yield from the roundwood was due entirely to the loss in yield of fatty acids. The loss in yield in the purchased slabwood chips was due predominantly to the loss in yield in fatty acids; however, there was, in addition, a small loss in resin acids, and a very small loss in unsaponifiables.

As for tall oil quality, by the end of 12 weeks of storage the acid number of tall oil from both roundwood and purchased chips had dropped below 160.

In correlating the yield of tall oil from the wood extractions with the yield of tall oil from the black liquor from digester cooks, it appears that on the average about 80% to 88% of the extracted tall oil can be found in the black liquor.

## Introduction

IN AN EFFORT to insure maximum possible tall oil production, a laboratory fact-finding program was initiated to secure more information and data concerning the fluctuations in tall oil production and the reasons behind these fluctuations. The study of age vs. tall oil yield for roundwood and purchased slabwood chips was a part of this overall program. Other aspects of this program, to be covered in additional reports, are the solvent extractions of weekly composites of wood to the digesters, the weekly determinations of tall oil potential to the smelters and the waste treatment plant, and the study of the effect of mixing hardwood and pine liquors on tall oil recoveries.

It is important to have the above data in order to determine how much tall oil is available, how

TABLE I
Tall Oil Aging Study
Comparison of Tall Oil Yield and Quality with Wood Storage Time
Basis Saponified Trichloroethane Extract of Wood Chips—Carolina

| Date | Wood Storage Time | % Loss in Tall Oil Yield | Yield—% on OD Wood | | | | Sap. No. | Acid No. | % Rosin Acids | (By Diff.) % Fatty Acids | % Unsaps | Tall* Oil Yield lb/Ton AD Pulp |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Tall Oil | Rosin Acids | Fatty Acids | Unsaps | | | | | | |
| Roundwood | | | | | | | | | | | | |
| 7/19/63 | Fresh | 0 | 2.89 | 1.05 | 1.56 | 0.28 | 183 | 167 | 36.8 | 54.1 | 9.6 | 123.8 |
| 8/ 9/63 | 3 wk | 1.7 | 2.84 | 1.14 | 1.43 | 0.27 | 172 | 161 | 40.8 | 50.4 | 9.3 | 121.7 |
| 8/30/63 | 6 wk | 14.9 | 2.46 | 0.92 | 1.30 | 0.24 | 174 | 165 | 37.8 | 52.9 | 9.8 | 105.4 |
| 10/11/63 | 12 wk | 7.3 | 2.68 | 1.09 | 1.30 | 0.29 | 171 | 157 | 40.6 | 48.5 | 10.9 | 114.8 |
| Purchased Slabwood Chips | | | | | | | | | | | | |
| 7/26/63 | Fresh | 0 | 3.09 | 1.24 | 1.63 | 0.22 | 191 | 172 | 40.0 | 52.9 | 7.1 | 132.4 |
| 8/ 2/63 | 1 wk | 17.5 | 2.55 | 1.13 | 1.26 | 0.16 | 184 | 177 | 44.2 | 49.5 | 6.3 | 109.3 |
| 8/16/63 | 3 wk | 33.7 | 2.05 | 1.05 | 0.84 | 0.16 | 180 | 169 | 51.1 | 41.2 | 7.7 | 87.8 |
| 9/ 6/63 | 6 wk | 56.6 | 1.34 | 0.83 | 0.39 | 0.12 | 170 | 163 | 61.7 | 29.4 | 8.9 | 57.4 |
| 10/18/63 | 12 wk | 64.4 | 1.10 | 0.67 | 0.32 | 0.11 | 189 | 156 | 61.4 | 28.8 | 9.8 | 47.1 |
| Regular Mill Chip Pile | | | | | | | | | | | | |
| 7/ 3/63 | Approx. 8 wk | — | 1.45 | — | — | — | — | — | — | — | — | 62.1 |
| 10/19/63 | 14 wk | — | 1.06 | 0.61 | 0.30 | 0.15 | 185 | 147 | 57.5 | 28.5 | 14.0 | 45.4 |

* The conversion from % on O. D. wood to No./ton A. D. pine pulp is based on a pulp yield of 42% or that 4285. lbs. O. D. wood equal one ton A. D. pulp.

much of the available is being collected, and whether the losses that are occurring can be prevented.

The aging study, showing the effect of aging on the yield as well as on the quality of tall oil, was carried out on a small scale. Limited by the number of people and amount of time and equipment available, it was not possible to deal with a large quantity of roundwood or chips, or to go into a large amount of detail as to different species or different seasons of the year for aging. Some of these points may be examined later. Likewise, time did not permit the study of the effect of aging on turpentine yield. There are still several points that are not clearly explained by the data, as will be observed on reading this report. These unusual results may be explained whenever further work is possible.

Since the actual aging of the roundwood and purchased chips was to be carried out at Carolina, it was decided to use solvent extractions as laboratory pulping facilities were not available at Carolina. However, before starting the aging study it was necessary first to develop a satisfactory procedure for solvent extraction of wood to determine tall oil yield. The technical details of this procedure will be covered in a separate report.

The attempt to correlate solvent extract and pulped yield of tall oil, in black liquor, was done at Knightsbridge in the pulp laboratory. One of the purposes of the weekly solvent extractions at Carolina is to establish the same correlation between solvent extract and pulped yield of tall oil over the different seasons of the year.

## Experimental Procedures and Data

### Roundwood

Four short-leaf pine trees were taken on July 18, 1963, from Duke Power Company land, Morganton District, in the Lake James area. The trees were all approximately the same size averaging 11.5 in. in diameter. They varied from 12 to 13 in. in diameter at the bottom to 8.5 to 9 in. in diameter at the top, or last bolt taken from the trees.

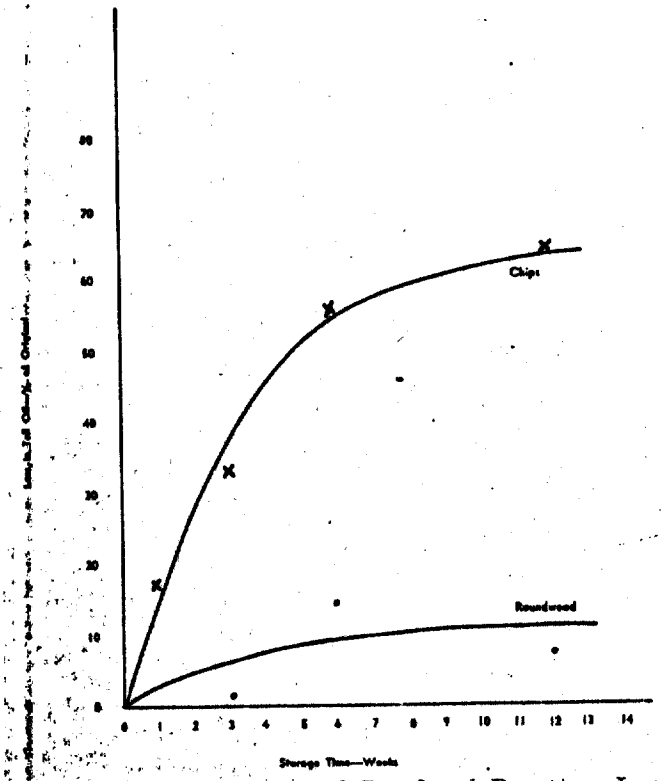


FIG. 1. Purchased Chips and Roundwood Percentage Loss in Tall Oil Yield vs. Storage Time.

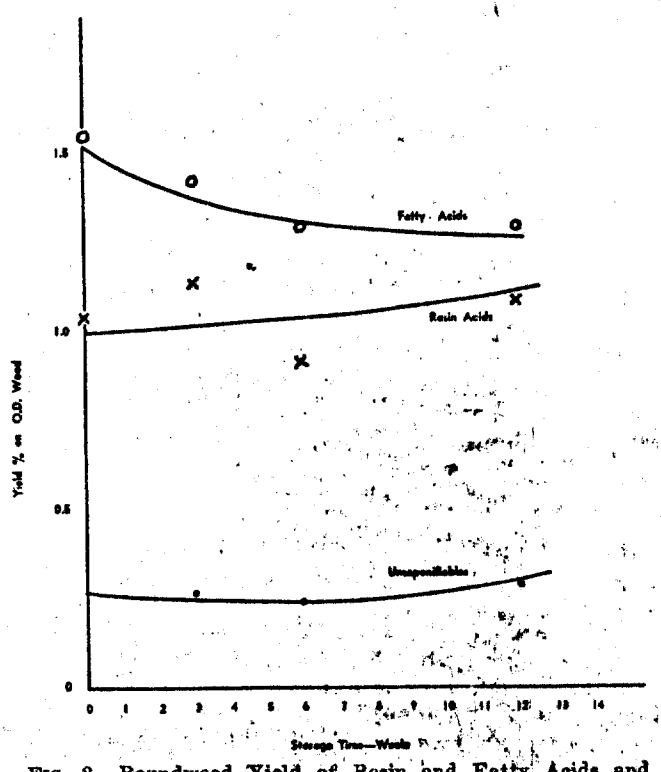


FIG. 2. Roundwood Yield of Rosin and Fatty Acids and Unsaponifiables vs. Storage Time.

Each tree was cut into six 5-ft-long logs. These logs were transported to Carolina immediately after harvesting and labeling. The four top and four butt logs were used in determining the difference between fresh-cut butt and top logs in tall oil content. The remaining logs were used in the aging study and were stored on the wood yard. Four age intervals were used: fresh cut, 3 weeks, 6 weeks, and 12 weeks. Each storage interval was tested on chips made from a composite of one log from each of the four trees, representing different heights in the tree. Before being chipped on one of the mill chippers, the logs were barked by hand. Afterwards, a representative sample of the chips was taken to the laboratory for solvent extraction and another sent to Knightsbridge for pulping.

#### Purchased Slabwood Chips

Purchased chips were obtained from the Gilkey Lumber Company on July 25, 1963. The chips were made from fresh slabwood from trees cut in the same area as the four pine trees mentioned above. The chips were obtained on the Carolina Wood Yard in a pile that measured 12.5 ft × 14.5 ft × 4 ft high. The pile contained 5,920 lb of chips. Five age intervals were used; fresh cut, 1 week, 3 weeks, 6 weeks, and 12 weeks. The samples for solvent extraction and pulping were always taken from about the center of the pile and at least halfway down into the pile.

#### Tall Oil from Solvent Extractions: Carolina

The chips were reduced to matchstick size in a Wiley Mill and then spread out to air dry in the laboratory for two days. The air-dried matchsticks were ground in a Wiley Mill to pass a screen with 1 mm diam opening. The moisture content of the sawdust was determined by drying 200 AD g overnight in an oven at 105C. Using a soxhlet unit, 400 AD g of sawdust were extracted for 18 hr with 1, 1, 2 trichoroethane. The sawdust was allowed to soak overnight in the solvent before and halfway through the extraction period. Following extraction the extract was concentrated first at atmospheric conditions and finally under vacuum. The concentrated extract was saponified by refluxing for 6 hr with 100 cc of 2 *N* alcoholic KOH, and then made up to 2 liters with distilled water. Aliquots were acidified 4 *N* $H_2SO_4$ and ether extracted with ethyl ether. Tests were run on the ether extracted material according to ASTM methods. Tall oil yield was determined from the weight of the ether extract and based on the OD weight of the wood extracted. [Note 1) The conversion from percent on OD wood to lb/ton AD pulp is based on a pulp yield of 42% or that 4.285 lb OD wood equals one ton AD pulp. Note 2) Trichloroethane for wood extractions, and petroleum ether for Buckeye test.]

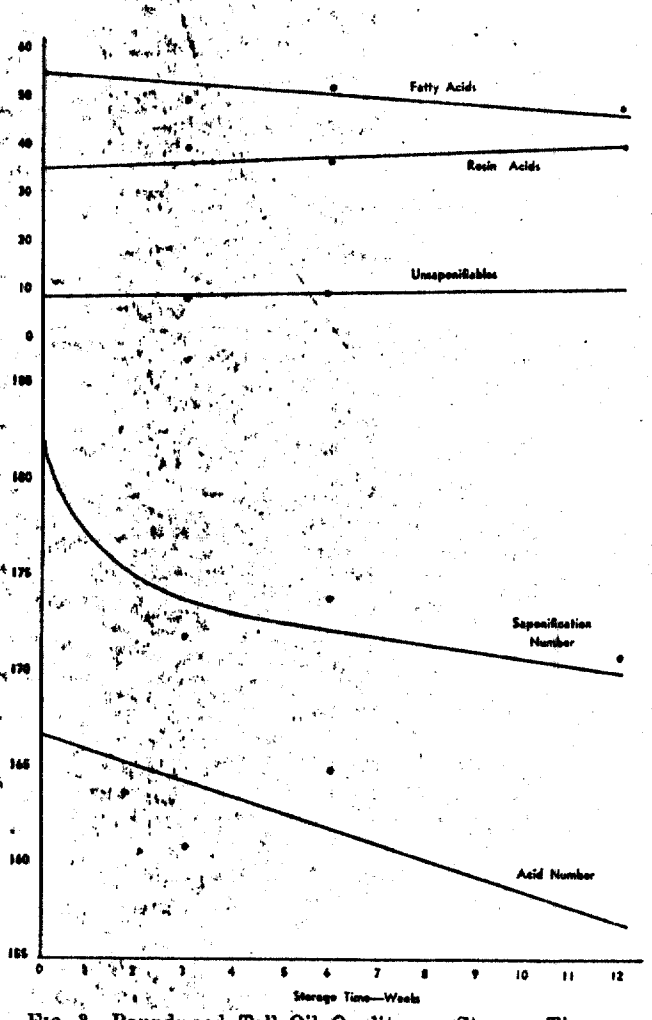


FIG. 3. Roundwood Tall Oil Quality vs. Storage Time.

#### Tall Oil from Digester Cooks: Knightsbridge

Part of the chip sample was cooked in a stationary indirectly-heated digester to a 16 PN. A charge of 2,000 OD g of full-sized chips was used. All of the black liquor was collected by thoroughly washing the pulp and a Buckeye test was run on the total collected liquor. The yield of tall oil was figured on a liquor solids basis and back-calculated to a wood basis knowing the total wood charged and the total solids in the black liquor.

The other part of the chip sample was extracted with trichloroethane using a procedure similar to that used at Carolina. However, the extraction results obtained were not used in the aging study due to the shipping time involved.

## Results

#### Roundwood Aging Study—Carolina (Table I)

The loss of tall oil yield over the 12-week aging period was very small, approximately 11% (Fig. 1.). The yield, based on OD wood, decreased from 2.9% to about 2.6% or from 124 lb tall oil per ton AD pulp Note 1 to about 110 lb/ton. This is taking into consideration that for some unexplained reason the yield was less at the end of 6 weeks than at the end of 12 weeks. However, the yield of tall oil from the fresh wood was somewhat lower than normally experienced. For comparison, the tall oil yield from the extractions of weekly composites of wood to the digesters for August through December 1963 averaged 3.4% or 144 lb/ton.

The yield of fatty acids over the 12 weeks decreased from 1.6% to 1.3%; while the yields of rosin acids and unsaponifiables increased only very slightly (Fig. 2), from 1.05% to 1.09% and from 0.28% to 0.29%, respectively.

As for the quality of the tall oil over the aging period, (Fig. 3) the saponification number, the acid number, and the per cent fatty acids decreased in value; the per cent rosin acids and the per cent unsaponifiables increased. The saponification number decreased from 183 to 171, the acid number from 167 to 157, and the per cent fatty acids from 54.1

TABLE II

Comparison of Tall Oil from Different Heights of a Fresh-Cut Pine Tree Basis Saponified Trichloroethane Extract of Wood Chips—Carolina

| Section of Tree | Yield—% on OD Wood | | | | Sap. No. | Acid No. | % Rosin Acids | (By Diff.) % Fatty Acids | % Unsaps. | Tall Oil Yield lb/Ton AD Pulp |
|---|---|---|---|---|---|---|---|---|---|---|
| | Tall Oil | Rosin Acids | Fatty Acids | Unsaps | | | | | | |
| Tops | 3.2 | 0.99 | 1.85 | 0.36 | 180 | 159 | 30.8 | 57.9 | 11.3 | 137.1 |
| Middle | 2.9 | 1.05 | 1.57 | 0.28 | 183 | 167 | 36.3 | 54.1 | 9.6 | 124.3 |
| Butts | 2.9 | 1.02 | 1.61 | 0.27 | 186 | 168 | 35.2 | 55.6 | 9.2 | 124.8 |

to 48.5. The percent rosin acids increased from 36.3 to 40.6, and the per cent unsaponifiables from 9.6 to 10.9.

**Tall Oil from Different Heights in Fresh-Cut Trees—Carolina (Table II, Fig. 4)**

The tops of the fresh-cut pine trees produced a higher tall oil yield than did the middle and butt sections. The tops yielded 3.2% or 137 lb/ton and the middle and butts 2.9% or 124 lb/ton.

The larger tall oil yield of the top sections was due to a larger yield of fatty acids and unsaponifiables. The tops had a yield of fatty acids of 1.85% as against 1.57% and 1.61% for the middle and butt sections, respectively; a yield of unsaponifiables of 0.36% as against 0.28% and 0.27%; and a yield of rosin acids of 0.99% as against 1.05% and 1.02%.

The top sections, in comparison with the middle and butt sections, which were about the same, had a lower saponification number, acid number and per cent rosin acids, and a higher per cent fatty acids and per cent unsaponifiables. The tops had a saponification number of 180 as compared to 183 and 186 for the middle and butt sections, respectively; an acid number of 159 as compared to 167 and 168; a per cent rosin acids of 30.8 as to 36.3 and 35.2; a per cent fatty acids of 57.9 as to 54.1 and 55.6 and a per cent unsaponifiables of 11.3 as to 9.6 and 9.2.

**Purchased Slabwood Chips Aging Study—Carolina (Table I)**

The loss of tall oil yield over the 12-week aging period was a dramatic 64%. One fourth of this total loss occurred within one week, one half by three weeks and seven eighths by six weeks, indicating a very sharp drop-off at first and then a gradual leveling off. The yield, based on OD wood, decreased from 3.1% all the way down to 1.1% or from 132 lb tall oil/ton A.D. pulp down to 47 lb/ton. Within one week the tall oil yield had dropped to 2.6% or 109 lb/ton; by three weeks to 2.1% or 88 lb/ton; and by six weeks to 1.3% or 57 lb/ton.

The yield of fatty acids over the 12 weeks decreased sharply, as did the total yield, from 1.63% to 0.32% (Fig. 5). The yield of rosin acids decreased also, but more gradually, from 1.24% to 0.67%. The yield of unsaponifiables decreased even more gradually from 0.22% to 0.11%. However, most of the losses in the above yields had occurred by six weeks. As shown in Figure 5 the fresh wood contained a greater yield of fatty acids than rosin acids. Within two weeks the yields were approximately the same, and afterwards the yield of fatty acids fell off below that of the rosin acids.

As for the quality of the tall oil over the 12-week aging period (Fig. 6), the saponification number, the acid number, and the per cent fatty acids decreased in value; the per cent rosin acids and per cent unsaponifiables increased in value. These changes were more pronounced than for the roundwood in keeping with the greater loss in yield. The high saponification number for the 12-week sample cannot be explained; however, as will be discussed later, the saponification number for the 14-week regular mill chip pile sample was also high. The saponification number decreased from 191 to 170 (six weeks), the acid number from 172 to 156, and the per cent fatty acids from 52.9 to 28.8. The per cent rosin acids increased from 40.0 to 61.4, and the per cent unsaponifiables from 7.1 to 9.8.

**Regular Mill Chip Pile—Carolina**

The regular mill purchased chip pile was sampled

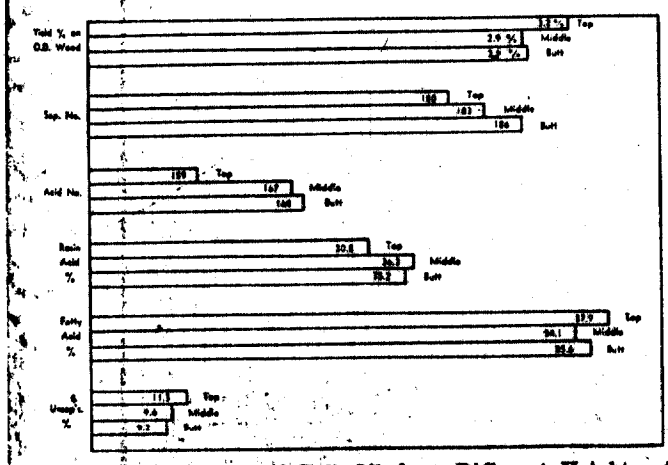


FIG. 4. Comparison of Tall Oil from Different Heights of a Fresh Cut Pine Tree.

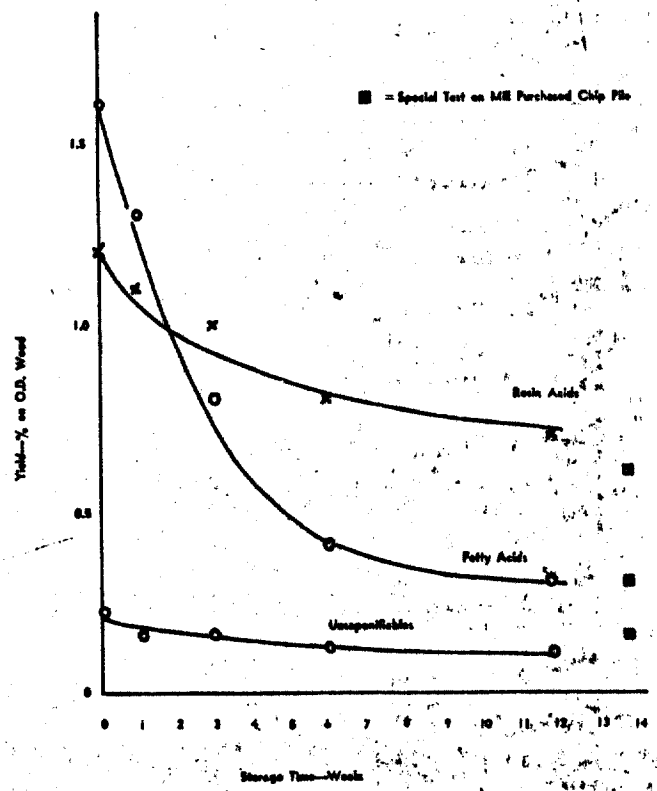


FIG. 5. Purchased Chips Yield of Rosin and Fatty Acids and Unsaponifiables vs. Storage Time.

TABLE III

Comparison of Tall Oil Yield from Wood with Tall Oil Yield after Pulping Basis Saponified Trichloroethane Extract of Wood Chips and Buckeye Test on Black Liquor from Digester Cooks—Knightsbridge

| Wood Storage Time | Trichloro. Extract Tall Oil Yield % on OD Wood | Black Liquor Tall Oil Yield % on OD Wood | % Yield from Black Liquor to Yield from Extract |
|---|---|---|---|
| **Roundwood** | | | |
| Fresh | 2.70 | 2.17 | 81% |
| 3 wk | 2.54 | 1.93 | 76 |
| 6 wk | 2.50 | 1.95 | 78 |
| 12 wk | 2.46 | 2.00 | 81 |
| | | | Av. 79.0% |
| **Purchased Chips** | | | |
| Fresh | 2.84 | 2.97 | 100+ % |
| 1 wk | 2.10 | 1.61 | 76 |
| 3 wk | 1.99 | 1.51 | 76 |
| 6 wk | 1.70 | 1.08 | 64 |
| 12 wk | 1.12 | 1.01 | 92 |
| | | | Av. 81.6% |

Carolina Special Weekly Tall Oil Potential Tests

| August Through December | Extracted Tall Oil Yield from Weekly Composites of Wood to Digesters | Total Tall Oil Potential: Production and Losses to Smelters and Waste Treat. Plant |
|---|---|---|
| 1963 | 143.8 lb Tall Oil/Ton AD Pulp | 126.2 lb/Ton 87.7% |

at the beginning and ending of the aging study. At the first sampling the pile contained about 470 cords and represented an approximate storage time of eight weeks. At the last sampling the pile held about 1500 cords and represented an approximate storage time of 14 weeks. Before any chips had been removed and sent to the pulp mill, the pile measured 190 ft x 180 ft x 30 ft high with tapered sides and contained about 2200 cords. The 14-week sample was taken at about the center of the pile and at a depth of about 24 ft.

As shown in Table I and Figures 5, and 6, the good agreement between the special aging study and the regular mill chip pile samples indicate that the small experimental chip pile simulated the regular storage conditions quite well.

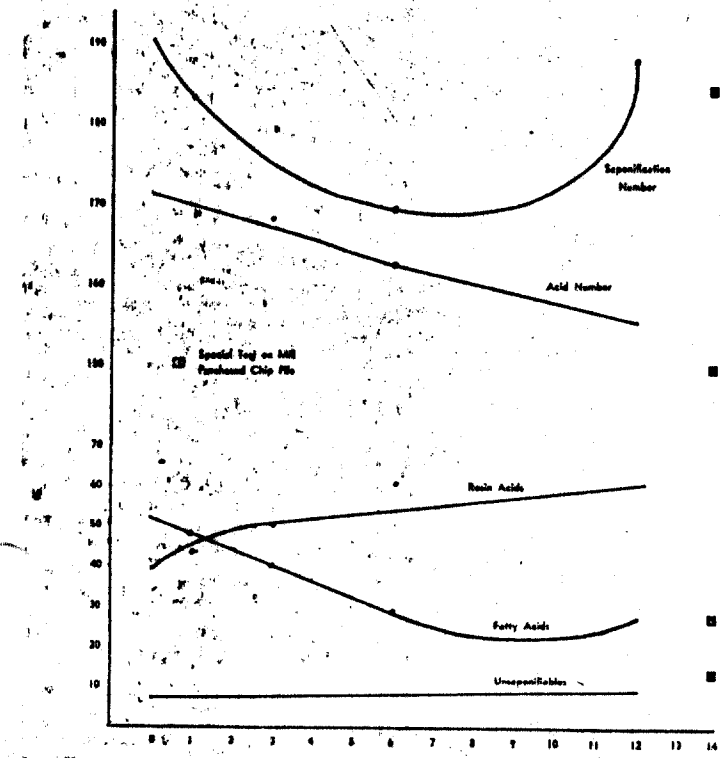


FIG. 6. Purchased Chips Tall Oil Quality vs. Storage Time.

**Extracted Yield vs. Pulped Yield—Knightsbridge (Table III, Fig. 7)**

The attempt to correlate the yield of tall oil from the wood extractions with the yield of tall oil from the black liquor from digester cooks has produced results that have not been completely conclusive. But it does appear that about 80% of the extracted tall oil can be found in the black liquor. There are some wide swings away from this value, primarily with the purchased chips, and these have not been satisfactorily explained at this time. Also, unexplained is the reason for the loss in tall oil yield on pulping as compared to the extracting of wood. It is not known whether it is because that two different solvents Note 2 were used, or that some of the material was actually lost on pulping. It is hoped to be able to resolve these questions with additional laboratory studies that are planned.

The Carolina special weekly tall oil potential tests give general support to the above mentioned 80% figure. For the five months, August through December 1963, the total tall oil potential averaged 126 lb tall oil per ton AD pulp. This consisted of tall oil production, and tall oil loss to smelters and waste treatment plant. For the same period the tall oil yields from the extractions of weekly composites of wood to the digesters averaged 144 lb/ton. Therefore, the relationship between solvent extract and pulped yield of tall oil gives a comparative figure of 88%

## Conclusions

In 12 weeks of storage time the roundwood lost approximately 11% in tall oil yield while for the same length of time the purchased chips lost 64%. Most of this loss in tall oil yield occurred within six weeks.

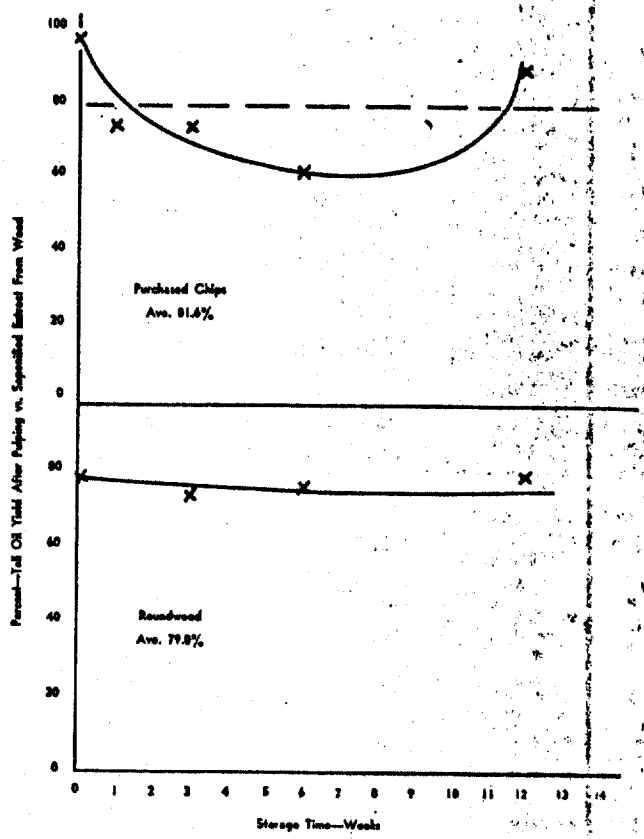


FIG. 7. Tall Oil Yield from Wood after Pulping.

For the purchased chips one half of the total loss in tall oil occurred within three weeks of storage. In fact the purchased chips lost more tall oil yield in one week than the roundwood in 12 weeks.

As far as tall oil yield is concerned, assuming a constant volume of chips in storage, there would be less sales revenue lost over a year's time by rotating the chip pile every six weeks than every week. This is due to the faster rate of tall oil loss in the first weeks of storage. It would be even more economical to leave the chips stored for longer periods, not exceeding the point at which pulp quality and yield would be affected. By accepting the loss of tall oil in this chip pile, a continuing loss at the faster initial rate would be prevented in the chips that otherwise would be rotated on a one to six weeks' basis.

As for tall oil quality, by the end of 12 weeks of storage the acid number of tall oil from both roundwood and purchased chips had dropped below the minimum specification of 160. This specification was set by Chemicals Division for Champion's Crude Dehydrated Tall Oil.

The loss in yield in the roundwood was due entirely to the loss in yield of fatty acids. The loss in yield in the purchased chips was due predominantly to the loss in yield in fatty acids; however, there was in addition a smaller loss in rosin acids, and a very small loss in unsaponifiables.

The loss in tall oil yield in the experimental purchased chip pile was substantiated by the loss in tall oil yield in the regular mill purchased chip pile.

In comparing the tall oil from the top sections of trees with the middle and butt sections, which were very similar, the tops had a little more yield, but a little poorer quality tall oil than the middle and butt sections; however, the differences were not too significant.

In attempting to correlate the yield of tall oil from the wood extractions with the yield of tall oil from the black liquor from digester cooks, it appears that on the average about 80% to 88% of the extracted tall oil can be found in the black liquor. The loss in tall oil yield on pulping as compared to the extracting of wood is unexplained at this time.

ACKNOWLEDGMENT

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## NUTRITIONAL ADJUNCTS

# Chick Edema Factor. III. Application of Microcoulometric Gas Chromatography to Detection of Chick Edema Factor in Fats or Fatty Acids

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**A rapid screening test for detecting chick edema factor in fats consists of adsorption chromatography of extracted unsaponifiables on alumina, followed by analysis of specific fractions by a microcoulometric gas chromatograph which is sensitive only to halogens. This chromatographic method appears to be more sensitive than the chick bioassay.**

**Toxic fat yielded gas chromatographic peaks with retention times relative to aldrin of .5 or more. All samples which failed to reveal those chromatographic peaks have been shown to be nontoxic in the chick bioassay.**

The widespread occurrence of chick edema disease in 1957 resulted in the deaths of millions of young chickens. The toxic materials causing this disease have been found to be chlorinated aromatic hydrocarbons, occurring in toxic fats in association with a large number of relatively nontoxic aromatic materials with similar chemical and physical properties. This paper describes a screening procedure for detection of such toxic fats. Specific fractions of unsaponifiable matter isolated from the fats are examined by using a microcoulometric gas chromatograph, an instrument which can detect submicrogram amounts of halogen. The presence of slow-eluting substances is an indication of the chick edema factor in the fat.

The precise structure of the substances causing chick edema disease have yet to be determined. Preliminary work on the detection, isolation, and characterization of the toxic agents was reported in 1959 by several laboratories (1–3). Subsequently,

Harmon and co-workers (4) isolated a toxic substance in crystalline form from a feed grade tallow. A private communication from Fishler of the same laboratory (5) disclosed that the crystalline substance contained about 47% chlorine.

Yartzoff and co-workers (6) isolated a crystalline halogen containing material that produced chick edema symptoms at 0.1 ppm in the diet from a sample of triolein. This triolein was toxic to monkeys, producing changes in the liver, kidney, pancreas, and other organs. More recently, Wootton and co-workers (7) isolated three compounds from a toxic fat which produced chick edema disease. Mass spectra of two of the compounds indicated a molecule which has a molecular weight of 391 and contains six chlorine atoms. Ultraviolet spectra were consistent with the concept that these materials are highly chlorinated aromatic compounds.

Ames and co-workers (8) and Firestone and co-workers (9) reported the occurrence of chick edema disease factor in oleic acid samples destined for human consumption. A food additive regulation[1] of the Food and Drug Administration now requires that food grade fatty acids be "free of chick edema or other toxic factor." At present, the detection and assay of chick edema factor in fats is carried out by a bioassay procedure (10–12) that requires 21 days to complete.

We observed that unsaponifiable matter from toxic fats contained a number of chlorinated components which had greater retention times than chlorinated pesticides when examined in a microcoulometric gas chromatograph;[2] our observation prompted this investigation of the use of microcoulometric gas chromatography for detecting the presence of chick edema factor in fats. Chick edema factor is presumed to be present if one or more gas chromatographic peaks with retention times relative to aldrin of 5 to 20 are found; its absence is presumed if analysis of the equivalent of 100 g of a fat or fatty acid fails to reveal the presence of these gas chromatographic peaks.

[1] Code of Federal Regulations, Title 21, Section 121.1070.

[2] Dohrmann Manufacturing Company, Palo Alto, Calif.

## METHOD

*Extraction of unsaponifiable matter* (modification of AOAC method 26.064(5)). — Reflux 111 g sample with 270 ml alcohol and 55 ml 50% (w/w) KOH for 1 hour. Transfer mixture to 2 L separator, rinsing flask with 325 ml $H_2O$, and add rinsings to separator. Add 300 ml petroleum ether, A.C.S. (redistilled, retaining cut with b.p. 40–60°C), and shake vigorously. Let layers separate, breaking emulsions that may have formed by adding 10 ml alcohol and swirling gently. Draw off lower layer, and transfer upper layer to another separator. Repeat extraction 3 times with 300 ml portions of petroleum ether and combine extracts. Wash extracts twice with 60 ml portions of $H_2O$ by swirling gently. Wash petroleum ether extracts first with 60 ml $H_2O$ and then with 60 ml of an alkaline dilute alcohol soln (dissolve 28 g anhydrous $K_2CO_3$ in 600 ml $H_2O$ and then add 400 ml alcohol), and repeat washings in same order. Wash extracts with 60 ml portions of $H_2O$ until neutral to phenolphthalein. Transfer extract to a 2 L erlenmeyer and dry by adding 20 g anhydrous $Na_2SO_4$, swirling vigorously, and letting the solution stand a half hour. Decant solution through a glass funnel, containing a pledget of cotton in the neck and holding 20 g anhydrous $Na_2SO_4$, into another 2 L erlenmeyer. Wash first erlenmeyer and funnel with three 10 ml portions of petroleum ether, transferring washings from the erlenmeyer through the funnel and into the filtered solution. Evaporate most of solvent on steam bath, and transfer extract to 100 ml tared fat flask containing several boiling chips. Evaporate solvent on steam bath and complete drying under a gentle current of air, or by evacuating flask to 0.5 cm of mercury while swirling on steam bath. Determine weight of unsaponifiable matter.

*Fractionation of unsaponifiable matter by alumina chromatography.* — To a chromatographic column, 25 mm o.d. × 300 mm long, fitted at the bottom with a coarse porosity fritted glass disk and Teflon stopcock, add redistilled petroleum ether, dried prior to use with anhydrous $Na_2SO_4$, until column is ⅔ full. Weigh 50 g aluminum oxide (Merck reagent, No. 71707), and transfer to column. Store the alumina in tightly closed bottle, and close bottle as soon as possible after weighing out portions for chromatography. Let alumina settle, and when air bubbles stop rising to the

surface of the solvent, place a disk of coarse filter paper on top of the alumina. Cover the disk with 20 g anhydrous $Na_2SO_4$. Drain the excess petroleum ether so that it is level with the upper surface of the $Na_2SO_4$.

Transfer unsaponifiable matter to the chromatographic column, using a total of 20 ml petroleum ether. Allow liquid level to fall so that it is just below the top of the $Na_2SO_4$. Elute sample with 400 ml portions of each of the following solvents (dried prior to use by shaking with anhydrous $Na_2SO_4$): Petroleum ether (fraction 1), 5% ethyl ether in petroleum ether (fraction 2), and 25% ethyl ether in petroleum ether (fraction 3). Collect eluates in 500 ml erlenmeyer flasks, add several boiling chips, and evaporate to small volume on steam bath. Transfer residues to tared fat extraction flasks, evaporate solvent, and weigh. Transfer to 2 g short style vials having screw cap with tin liner, and evaporate solvent.

*Microcoulometric gas chromatography.*—Dissolve 4 g silicone grease (Dow Corning High Vacuum Grease) or Dow Corning DC 200 silicone fluid (12,500 centistokes) in 200 ml chloroform on steam bath. Add 16 g acid-washed Chromosorb W (Johns-Manville Co.), and stir continuously until most of solvent evaporates (about half an hour). Let stand on steam bath 1 hour, and place in vacuum oven at 50 C overnight to remove residual solvent.

Pack the coated Chromosorb W into a 3' length of 0.25" o.d. aluminum tubing plugged at one end with glass wool, using a Burgess Vibratool. (Two 3' columns may be prepared from 20 g coated Chromosorb W.) Add a plug of glass wool to the open end of the column and bend it into a tight spiral, using a 3" diameter mandril. Condition the column at 275°C for 48-72 hours, passing nitrogen through at 20 ml per minute.

Prepare a $1.00 \times 10^{-3}$% solution (10 mg/L.) of aldrin in hexane or benzene and chromatograph 100 µl portions in a Dohrmann microcoulometric gas chromatograph at 246-248 C, using a nitrogen flow rate of 50-100 ml per minute so that aldrin elutes in 2.3-3 minutes. Use the 128 ohm range setting.[5] Determine area of aldrin peak by triangulation, or with a disc chart or electronic integrator installed on the strip chart recorder, and calculate recovery of aldrin using the following equation (applicable to chlorinated compounds):

$$\mu g \text{ Aldrin} = (\text{peak area, in.}^2) \times [\text{recorder sensitivity (min./in.)(mv/in.)}] \times (35.5 \text{ g/eq.}) \times (60 \text{ sec./min.}) \times (10^6 \mu g/g)(10^3 \text{v/mv}) (10)/(\text{sensitivity range, ohms}) \times (\% \text{ chlorine in compound}) \times (96{,}500 \text{ coulombs/eq.}).$$

For a 0.1 mv/in. recorder sensitivity, 2 min./in. chart speed, and 12.8 ohms sensitivity range resistance, the equation above reduces to:

$$\mu g \text{ Aldrin} = (\text{area} \times 34.5)/\% \text{ chlorine.}$$

The number of strokes of a disc chart integrator coupled to a chromatography recorder[6] equivalent to each square inch of area can be determined as follows: (*a*) Remove the fuse from the strip chart amplifier; (*b*) move the pen upscale on the strip chart a known distance from the baseline; (*c*) run the chart a known distance; and (*d*) divide the calculated area (height × distance traversed by the pen) by the number of strokes obtained.

By using the formula calculated as described above, a recovery of at least 70% of the aldrin injected should be obtained.

Dissolve fractions 2 and 3 from alumina chromatography separately in benzene to give 100 µl solution, and chromatograph each solution in the Dohrmann instrument. (For analysis of more than about 60 mg of each fraction, approximately 50% benzene solutions of up to 250 µl volume should be prepared and injected. Do not inject more than 125 mg material into column). First chromatograph 1/10 of the fraction, and if no chromatographic peaks with $R_A = 5$ or greater are observed, chromatograph the remaining 9/10 of the fraction (equivalent to 100 g starting sample). Chromatograph a portion of aldrin before each sample, and calculate $R_A$ value (retention time relative to aldrin) of each peak in the sample chromatogram, using a millimeter rule to measure retention times. Record $R_A$ of gas chromatographic peaks in the range $R_A = 5$–20. Peaks in this range are indicative of the presence of chick edema factor. The presence of broad bands with no definite peaks is not indicative of the presence of chick edema factor.

(*Note:* Types of samples which are found from experience to be generally free of components characteristic of toxic fats may be examined as described above in 100 g portions, the sample saponified by refluxing with 240 ml alcohol and 50 ml 50% (w/w) KOH, and all of each of the polar alumina fractions gas chromatographed.)

[5] This setting will have a resistance of 12.8 ohms when the chromatograph is used with a 1 mv strip chart recorder.

[6] Minneapolis-Honeywell Model Y 153X (Minneapolis-Honeywell Regulator Co., Philadelphia, Pa.), or equivalent.

## Results and Discussions

*Relative Retention Times of Chlorinated Pesticides and Chlorinated Materials from Toxic Fats.*—A number of chlorinated pesticides and several chlorinated materials isolated from toxic fats were chromatographed in the Dohrmann instrument at 248°C with the 3′ column. Retention times relative to aldrin ($R_A$) are shown in Table 1. The pesticides are representative of the whole range of retention times displayed by chlorinated pesticides. A toxic factor isolated from triolein (6), an inactive analogue, and a concentrate prepared from a toxic fat, all yielded chromatograms with peaks of $R_A$ = 5 or greater whereas the pesticide peaks were all less than $R_A = 4$. The toxic factor from triolein as well as the toxic fat concentrate produced chick edema when fed to young chicks at a level of 0.1 ppm in the diet.

**Table 1. Relative retention times of chlorinated pesticides and materials isolated from toxic fats**

(3 foot, ¼ in. diameter column, 20% silicone grease, 80% Chromosorb W; carrier gas flow rate, about 60 ml/min.; column temperature, 248°C; injection block temperature, 270°C)

| Sample | Retention Time *vs.* Aldrin ($R_A$) |
|---|---|
| Chlordane | 1.0 |
| Heptachlor | 0.9 |
| Kepone | 2.2 |
| Mirex | 3.6 |
| Strobane | 0.5–3.5 |
| Tedion | 3.4 |
| Toxaphene | 0.6 3.8 |
| Toxic factor from triolein | 5.0 |
| Inactive analogue from triolein | 9.0 |
| Concentrate from a toxic fat | 2.3, 3.6, 5.4 |

The chick edema-producing factors isolated by Wootton and co-workers (7) had retention times relative to methyl arachidate of 1.17, 3.02, and 3.17 when chromatographed at 250°C on a 20% silicone column. Since aldrin elutes twice as fast as methyl arachidate under these conditions, it would be expected that these toxic factors would have $R_A$ values of about 2.4, 6.0, and 6.3 at 250° C on silicone columns. When a low-melting inactive isomer[5] having the same retention time ($R_A$ = 6.0) as one of the toxic factors was chromatographed in the Dohrmann instrument, the following $R_A$ values were obtained at 246°, 248°, and 250°, respectively: 6.6, 6.4, and 6.2.

*Preliminary Analysis of a Group of Toxic and Nontoxic Fats. Microcoulometric Analysis of Unsaponifiable Matter Without Prior Fractionation on Alumina.*—A group of 7 toxic and 7 nontoxic fats were examined initially. The fats are described in Tables 2 and 3, respectively. Presence of chick edema disease was determined by bioassay (2) using a special basal ration. Organic halogen in the unsaponifiable matter (assumed to be chlorine) was determined by microcoulometric gas chromatographic analysis of 50 mg portions of unsaponifiable matter without prior fractionation by alumina chromatography. Both toxic and nontoxic fats contained widely variable amounts of unsaponifiable matter and organic chlorine. Sources refer to individual manufacturers. Gas chromatograms of unsaponifiable matter from two of the toxic fats (Nos. 1 and 2) show peaks with $R_A$ values of 14. Gas chromatograms of unsaponifiable matter from the

**Table 2. Data on toxic fats**

| Component | Manufacturer | % Unsaponifiable Matter | Organic Cl in Unsaponifiable Matter, ppm |
|---|---|---|---|
| 1. Tallow acids, still distillate | 1 | 18.3 | 10 |
| 2. Tallow acids, still distillate | 1 | 14.8 | 47 |
| 3. Tallow acids, still distillate | 2 | 10.1 | 14 |
| 4. Tallow acids, still distillate | 3 | 2.9 | 25 |
| 5. Tallow acids, still residue | 1 | 4.5 | 2000 |
| 6. Tallow | 2 | 5.1 | 39 |
| 7. Fat from broiler feed | 4 | 2.5 | 8000 |

[5] Supplied by Dr. N. R. Artman, Procter and Gamble Co., Cincinnati, Ohio.

**Table 3. Data on nontoxic fats**

| Component | Manufacturer | % Unsaponifiable Matter | Organic Cl in Unsaponifiable Matter, ppm |
|---|---|---|---|
| 1. Cottonseed oil (CSO) | 8 | 0.6 | 80 |
| 2. Cottonseed oil | 8 | 0.5 | 13 |
| 3. CSO foots, still residue | 6 | 12.1 | 5 |
| 4. CSO fatty acids | 2 | 0.4 | 31 |
| 5. Vegetable oil foots | 5 | 2.2 | 28 |
| 6. Tallow fatty acids, still residue | 6 | 2.2 | 7 |
| 7. Corn oil | 7 | 0.6 | 150 |

other toxic fats and from nontoxic fats obtained without prior fractionation by alumina chromatography were similar: most of the organic halogen eluted in 2–4 minutes, and there were no peaks with $R_A$ values greater than 3. Since components with $R_A$ values of 5 or more are usually present in the unsaponifiable matter of toxic fats at very low levels, a concentration step by alumina chromatography is necessary prior to microcoulometric analysis.

*Fractionation of Unsaponifiable Matter on Alumina Prior to Microcoulometric Analysis.*—The 7 toxic and 7 nontoxic fats were then analyzed by procedures essentially as described above. At first, 6-foot, and later, 3-foot chromatographic columns were used in the microcoulometric gas chromatograph. With shorter columns, faster elution permitted analysis of 12 samples each working day, and results were comparable to those obtained with the conventional 6-foot columns. Because 10–100 mg portions of sample were repeatedly injected, all components of the microcoulometric gas chromatograph were cleaned every 2–4 weeks as required. Gas chromatographic columns were replaced each 1–2 months. In cases where samples contained large amounts of unsaponifiable matter, larger alumina columns were used for the column chromatography so that the ratio of alumina to unsaponifiable matter was at least 20 to 1. In these cases appropriately larger amounts of eluting solvents were also used.

Fractions obtained by adsorption chromatography on alumina of unsaponifiable matter were analyzed by microcoulometric gas chromatography. Gas chromatograms of polar fractions from toxic fats (eluted with 5% and 25% ethyl ether in petroleum ether) all showed peaks with $R_A$ values greater than 5. No peaks with $R_A$ values greater than 5 were found in gas chromatograms of these fractions from the nontoxic fats.

Portions of alumina fractions 2 and 3 equivalent to only about 50 g of the nontoxic still residues (Table 3, samples 3 and 6) were chromatographed in the Dohrmann instrument because of the presence of a large amount of crystalline material in these fractions. The infrared spectrum of this material (isolated by recrystallizing from petroleum ether) resembled that of dipalmitone. An additional cleanup procedure must be developed for routine analysis of 100 g samples of such still residues. Additional examination of unsaponifiables from 2000 g portions of three nontoxic vegetable oils (Table 3, samples 1, 2, and 7) failed to reveal chromatographic peaks with $R_A$ values greater than 3.

**Table 4. Slow-eluting peaks in microcoulometric gas chromatograms from analysis of toxic fats**

| No. | Toxic Fat | Manufacturer | $R_A \geqq 5$ |
|---|---|---|---|
| 1 | Tallow acids, still distillate | 1 | 6, 9, 12, 21 |
| 2 | Tallow acids, still residue | 1 | 6, 9, 10, 14 |
| 3 | Tallow acids | 1 | 6, 9, 10, 18 |
| 4 | Tallow | 2 | 6, 9, 12 |
| 5 | Tallow acids, still distillate | 2 | 6, 9, 10, 18 |
| 6 | Tallow acids | 3 | 6, 10 |

Table 4 lists the slow-eluting peaks found in gas chromatograms from 6 of the 7 toxic fats. Figure 1 shows chromatograms of alumina fraction 3 from three still distillates, each of which was obtained from a different manufacturer of commercial fatty acids. A similar pattern of slow-eluting peaks sug-

gests that a common complex contaminant may be responsible for the presence of chick edema factor in fats. Each peak probably represents a complex mixture of closely related compounds. In fact, when polar alumina fractions from several toxic fats were further fractionated by additional column chromatography on alumina, such purification often resulted in partial resolution of the corresponding peaks into at least 2 components.

The fat from a chick edema-producing sample of broiler feed (toxic sample 7) contained over 400 ppm chlordane, and this sample required special treatment because large quantities of chlordane eluted in alumina fractions 2 and 3. Although the $R_A$ of chlordane = 1, the large amounts present produced overloaded chromatograms which interfered with gas chromatographic detection of other components. A portion of combined fractions 2 and 3 was molecularly distilled in a "cold finger" pot still for 2 hours at 85°C and 50 $\mu$ pressure. The chlordane was volatile under these conditions and was eliminated from the residue which was analyzed in the Dohrmann instrument. Peaks with $R_A$ values greater than 5 were then found in the chromatograms.

These results indicated that alumina chromatography of unsaponifiable matter followed by microcoulometric gas chromatography of appropriate fractions might be used as a screening procedure to detect chick edema factor in fats and fatty acids. Additional work on cleanup procedures is required before this technique can be applied routinely to examination of 100 g samples of low-grade fats, such as still residues containing large amounts of material that elute in alumina fractions 2 and 3.

*Effect of Alumina Activity and Column Dimensions on Adsorption Chromatography.*—Alumina activity was found to affect the rate of elution of substances from toxic fats which are responsible for the gas chromatographic peaks of $R_A = 5$ and greater which are characteristics of toxic samples. Various batches of Merck alumina used for this work were found to vary in activity from

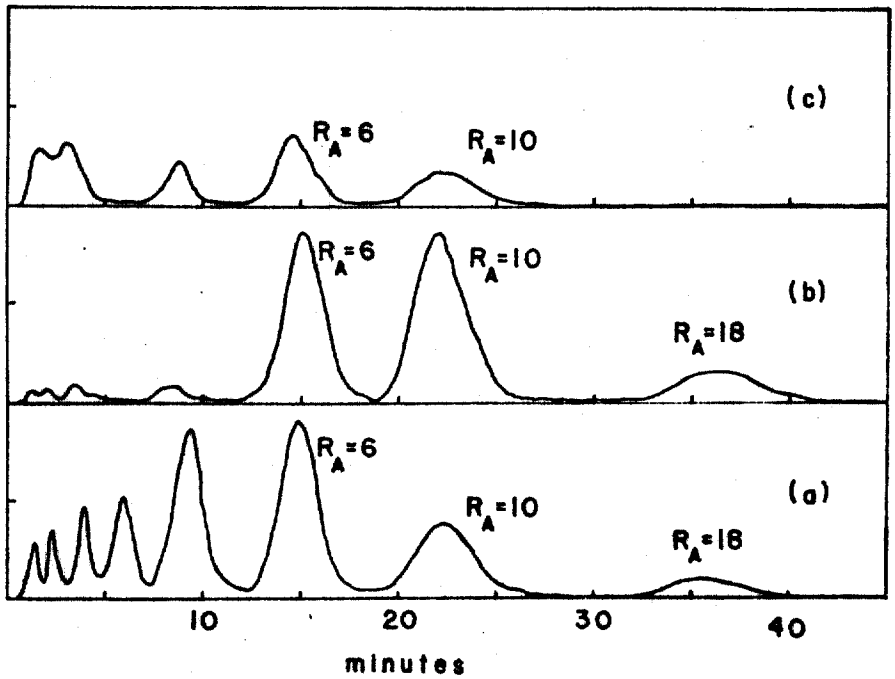


*Fig. 1*—Microcoulometric gas chromatograms of alumina fraction 3 isolated from toxic batch still distillates obtained from 3 manufacturers of commercial fatty acids. The fractions were isolated from (a) 5 g, (b) 5 g, and (c) 20 g of fat.

Brockmann activity I to activity II. Activities were determined by observing the rate of travel of solutions of specific pairs of azo dyes (13, 14). Using activity I alumina, unsaponifiable matter from toxic fat or from toxic fat added to USP cottonseed oil was eluted from the columns so that the characteristic slow-eluting peaks were found in gas chromatograms of alumina fraction 3. With activity II alumina, these peaks were found in chromatograms of alumina fraction 2.

Generally, the alumina used in this work was not standardized; it required gas chromatographic analysis of alumina fractions 2 and 3. Standardization of the alumina should permit elution of the slow-eluting compounds in one fraction, reducing the number of samples required for gas chromatography. For example, Merck alumina heated 48 hours at 200°C had a Brockmann activity 1, and all the slow-eluting peaks from several toxic fats examined were found in chromatograms of fraction 3. Work is continuing on a procedure for standardizing alumina in a simple and reproducible manner.

Column dimensions also were found to affect the elution of characteristic substances from toxic fats. When activity I alumina was used, these substances were eluted in fraction 3 from a 25 × 300 mm column, whereas they eluted in fraction 2 from a 30 × 300 mm column.

*Effect of Column Temperature and Flow Rate on Gas Chromatography of Slow-eluting Components of Toxic Fats.*—Studying the gas chromatographic behavior of chlorinated pesticides, Burke and Johnson (15) found that varying the column temperature and/or the carrier gas flow rate resulted in variations of relative retention times of the pesticides. Similar variations in relative retention times were observed with the slow-eluting components of toxic fats. $R_A$ values, however, were affected more by variations in temperature than in flow rate. A toxic substance isolated from triolein (6) had the following $R_A$ values at 246°, 250°, and 252°C: 5.6, 4.9, and 4.7. An inactive analogue isolated from the sample had the following $R_A$ values at these temperatures: 9.7, 8.0, and 7.8.

Because of the design of the microcoulometric gas chromatograph used for this work, whereby oven temperatures are controlled only by a variable transformer, line voltage fluctuations result in continuous variation of column temperature. A variation of ± 1°C within a 1–2 hour period is the best stability to be expected. Even with these variations, however, the instrument is suitable for detection of slow-eluting materials in toxic fats because of the large difference in retention times between these slow-eluting materials and the chlorinated pesticides and other fast-eluting chlorinated materials found in all fats examined.

*Analysis of Bioassay Collaborative Samples.*—A recent collaborative study of the AOAC bioassay method for detection of chick edema disease (12) indicated that the lower limit of sensitivity was obtained with a test sample containing 1.56% of a toxic fat in USP cottonseed oil. One hundred g portions of this sample, the toxic fat, and the original cottonseed oil were analyzed. Extracted unsaponifiables were chromatographed on 50 g alumina as described above, and the 5% and 25% ethyl ether eluates (fractions 2 and 3) were gas chromatographed. Chromatograms of fraction 2 from the cottonseed oil without and with added toxic fat are shown in Figs. 2 and 3, respectively. Peaks with $R_A$ values greater than 1.5, including the $R_A = 6$ and $R_A = 10$ peaks, are due to the toxic fat. A chromatogram of alumina fraction 2 from 100 g of test sample containing 0.78% toxic fat in

**Table 5. Analysis of USP cottonseed oil containing various levels of added toxic fat; microcoulometric gas chromatography of alumina fraction 2**

| Toxic Fat Added, % | Disc Integrator Response (No. of Pen Strokes) | |
|---|---|---|
| | $R_A = 6$ | $R_A = 10$ |
| 0.00 | 0 | 0 |
| 0.78 | 6 | 2 |
| 1.56 | 14 | 6 |
| 3.12 | 32 | 13 |
| 4.68 | 32 | 12 |
| 6.24 | 47 | 28 |

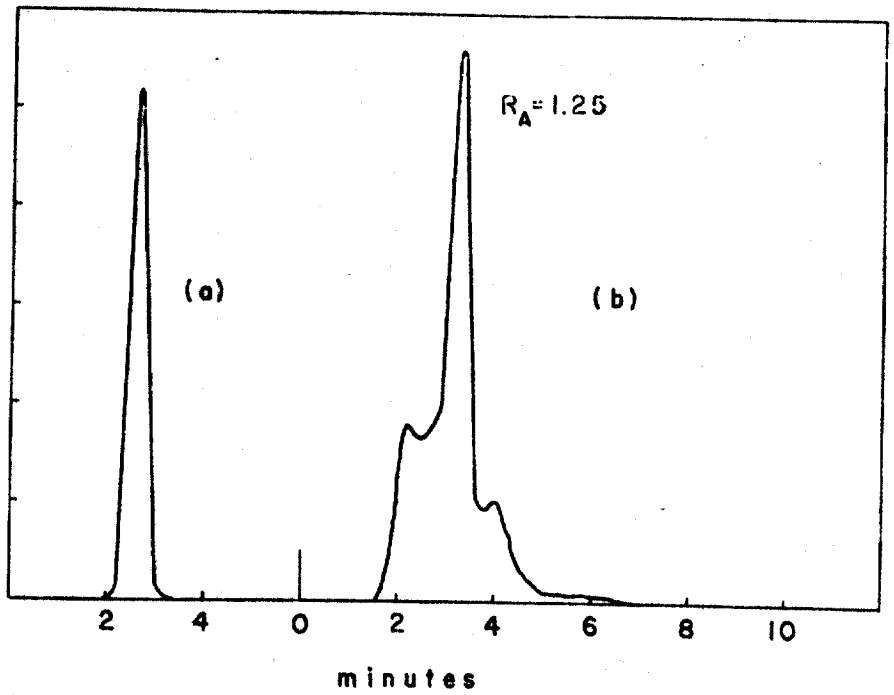


*Fig. 2*—Microcoulometric gas chromatograms of (a) aldrin standard (1 μg), and (b) alumina fraction 2 from USP cottonseed oil without added toxic fat.

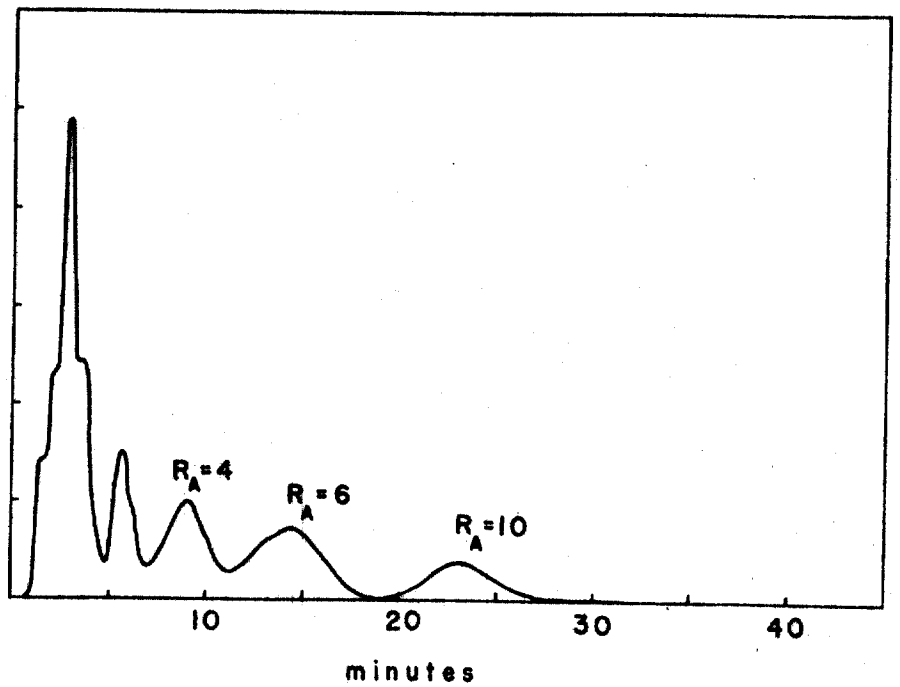


*Fig. 3*—Microcoulometric gas chromatogram of alumina fraction 2 from USP cottonseed oil containing 1.56% toxic fat.

the USP cottonseed oil is shown in Fig. 4. The $R_A = 6$ and $R_A = 10$ peaks can still be definitely detected at this level of toxic fat, which was half of that found to be at the lower limit of detection of the AOAC bioassay.

Samples of USP cottonseed oil containing various levels of added toxic fat up to 6.24% were analyzed. The disc integrator response of the $R_A = 6$ and $R_A = 10$ peaks are shown in Table 5. The integrator response (number of pen strokes) is approximately proportional to the level of added toxic fat in the cottonseed oil. Each stroke is equivalent to about 0.05 μg of organic halogen.

*Analysis of Commercial Oleic and Stearic Acids and Derivatives.*—Twelve food grade oleic acids were examined by the chromatographic method. The examination of 9 of these samples for chick edema toxicity was reported earlier (9). Gas chromatograms of alumina fractions from a nontoxic and a toxic acid are shown in Figs. 5 and 6, respectively. Chromatographic peaks of $R_A = 6$ and greater from the toxic sample are shown in Fig. 6. These peaks were present in alumina fractions 2 and 3. The "peak" with $R_A = 6$ in the chromatogram from alumina fraction 1 is believed to be an artifact due to overloading of the coulometer.

Results of analysis of the 12 oleic acids, compared with the chick bioassay using a special basal ration (2), are shown in Table 6. Hydropericardium activity, the primary index of the presence of chick edema factor, was estimated as described by Firestone and co-workers (9). Six samples were positive by both the biossay and chromatographic methods. One sample (No. 9) was negative by the regular bioassay when fed at the usual level of 16% in the diet. However, a positive response was obtained when extracted unsaponifiable matter was fed at a level equivalent to 6 times that present in the normal test diet.

Sample 10 was negative by the chick bioassay, but gave a weak positive response by the chromatographic method. A small chromatographic peak with $R_A = 10$ was obtained from alumina fraction 2. In comparing bioassay activity and peak areas of slow-eluting components of the other toxic oleic acids, it would be expected that the level of chick edema factor in sample 10

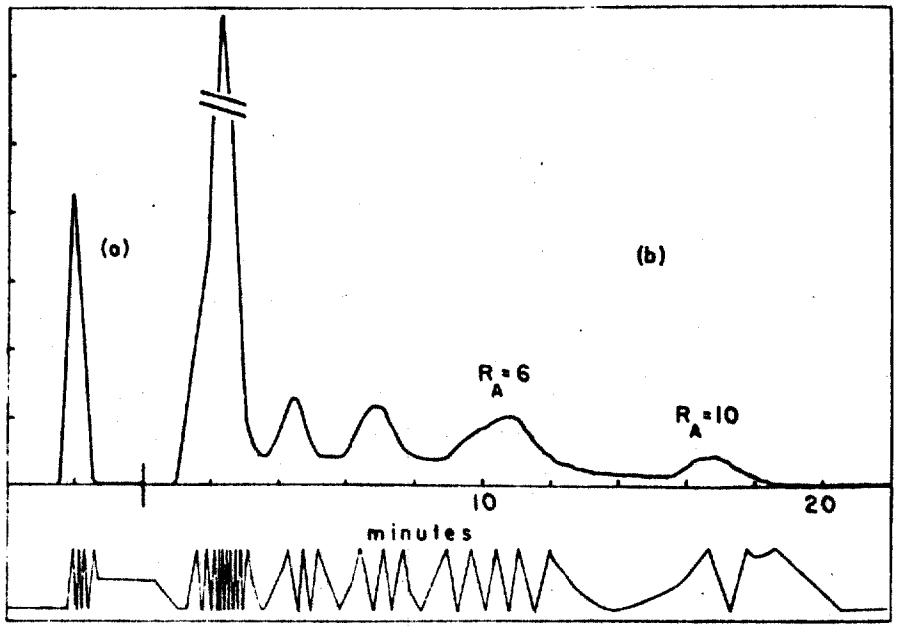


*Fig. 4*—Microcoulometric gas chromatograms of (a) aldrin standard (1 μg), and (b) USP cottonseed oil containing 0.78% toxic fat. Also shown are disc integrator pen strokes.

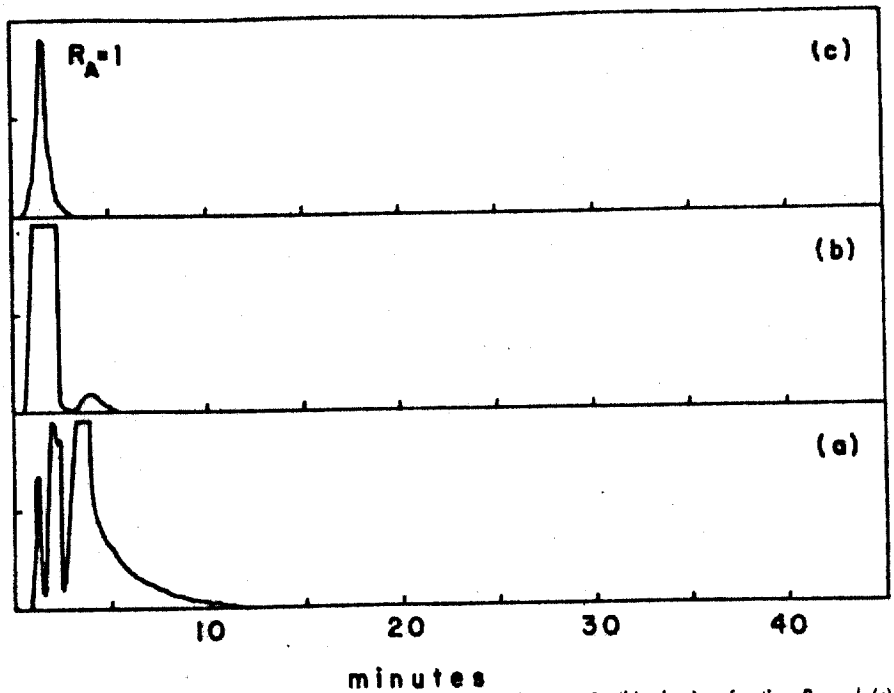


*Fig. 5*—Microcoulometric gas chromatograms of (a) alumina fraction 1, (b) alumina fraction 2, and (c) alumina fraction 3 from a non-toxic commercial oleic acid.

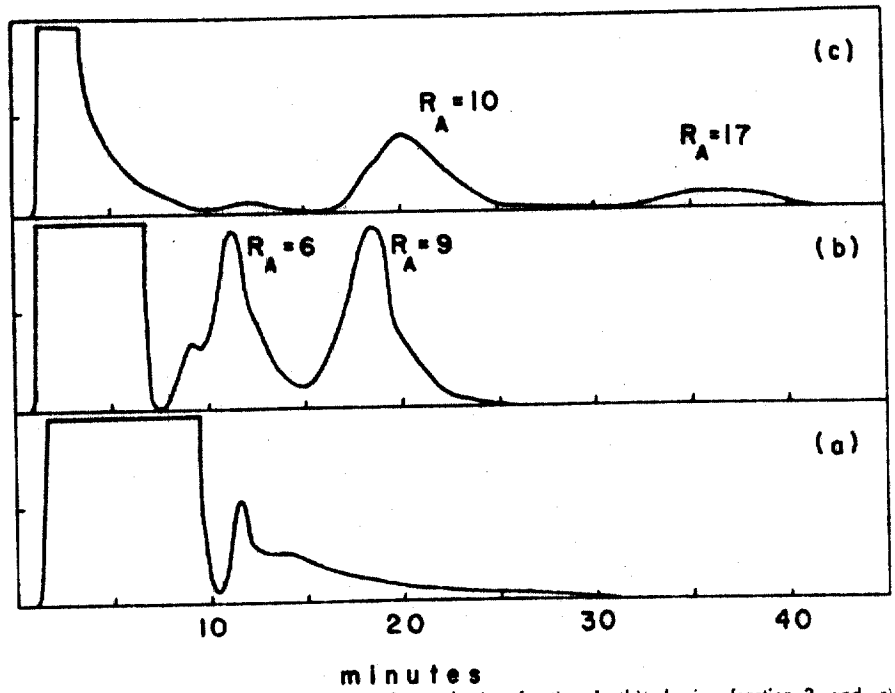


*Fig. 6*—Microcoulometric gas chromatograms of (a) alumina fraction 1, (b) alumina fraction 2, and (c) alumina fraction 3 from a toxic commercial oleic acid.

**Table 6. Analysis of commercial oleic acids**

| Sample No. | Bioassay: Hydropericardium Activity (9) | Chromatographic Analysis, Relative Peak Area (Integrator Strokes[a]) | | |
|---|---|---|---|---|
| | | $R_A$ = 5-6 | $R_A$ = 9-10 | $R_A$ = 15-18 |
| 1 | +0.2 | 74 | 150 | 9 |
| 2 | +3 | 340 | 90 | 8 |
| 3 | +0.3 | 98 | 196 | 104 |
| 4 | +0.1 | 118 | 156 | 9 |
| 5 | +0.2 | 46 | 88 | 14 |
| 6 | — | | | |
| 7 | — | | | |
| 8 | — | | | |
| 9 | +0.1[b] | 106 | 105 | 25 |
| 10 | — | | 0.6 | |
| 11 | — | | | |
| 12 | — | | | |

[a] Each stroke is equivalent to about 0.05 μg organic halogen.
[b] Unsaponifiable matter fed at a level equivalent to 6 times that present in the normal test diet.

might be below that detectable by the bioassay.

In comparing hydropericardium activity and gas chromatographic response (relative peak area), no parallel relationship was found between the bioassay response of the toxic oleic acids and the chromatographic response. It should be emphasized, however, that the slow-eluting compounds in toxic samples represent both toxic and relatively nontoxic materials, and most of the gas chromatographic response is probably due to relatively nontoxic substances. Nevertheless, microcoulometric gas chromatographic detection of slow-eluting compounds appears to be an effective screening tool for segregating questionable samples and diverting them to nonedible uses or for further testing by bioassay.

The gas chromatographic response of the $R_A$ = 9 peak present at widely different levels in 2 oleic acids was checked by analysis of individual samples on different days, using different gas chromatographic columns for each run. Results are shown in Table 7. Sample 1 contained a low level of material responsible for the $R_A$ = 9 peak. The differences in integrator response are due both to variation in coulometer response and to inaccuracies of disc integrator response at low halogen levels. The larger amounts of material in sample 2 responsible for the $R_A$ = 9 peak were distributed in alumina fractions 2 and 3 to varying extents depending upon the alumina activity. The total integrator response from both alumina fractions was fairly constant, however, varying from 136 to 169 integrator pen strokes.

In addition to the oleic acids, a number of derivatives of oleic acid (triolein, glycerol monooleate, etc.) were analyzed. Nine of ten samples were negative by both the bioassay and chromatographic analysis. One toxic sample, a triolein, gave gas chromatograms with peaks of $R_A$ = 6, 10, and 19.

Ten stearic acids and derivatives examined were negative by the bioassay and chromatographic method.

*Analysis of Animal and Vegetable Fats and Commercial Vegetable Oil Fatty Acids.*—Fifteen animal and vegetable fats were examined. Three toxic animal tallows were positive by the chromatographic method.

**Table 7. Response[a] of $R_A$ = 9 peak from 2 oleic acids**

| Run No. | Sample 1 | | Sample 2 | | |
|---|---|---|---|---|---|
| | Fraction 2 | Fraction 3 | Fraction 2 | Fraction 3 | Total |
| 1 | 0.2 | 0 | 20 | 116 | 136 |
| 2 | 0.8 | 0 | 96 | 48 | 144 |
| 3 | 0.6 | 0 | 168 | 0.5 | 169 |
| 4 | 5.0 | 0 | 100 | 50 | 150 |

[a] Disc integrator response in number of disc integrator pen strokes.

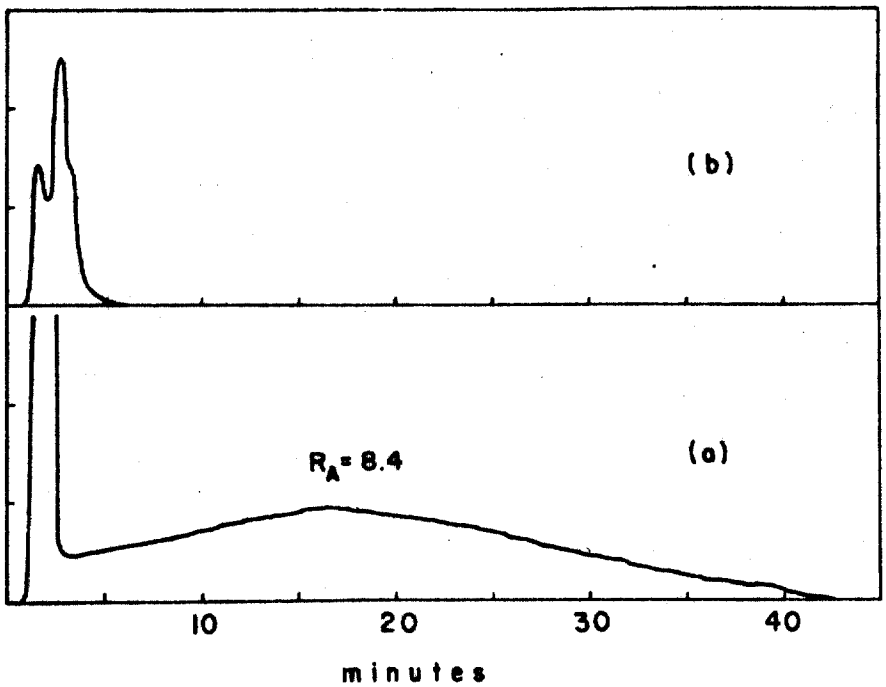


*Fig. 7*—**Microcoulometric gas chromatograms of (a) alumina fraction 2, and (b) alumina fraction 3 from a growth-depressing cottonseed oil which did not produce chick edema disease.**

Ten vegetable fats (including cottonseed oil, corn oil, peanut oil, safflower oil, and soybean oil) were examined, and were negative by the bioassay and chromatographic method. Chromatograms from several of the vegetable oils showed broad bands with no definite peaks. Chromatograms of alumina fractions 2 and 3 from one of these samples, a USP cottonseed oil, are shown in Fig. 7. The broad band in chromatogram (a) is typical of that found in the other oils. This sample depressed the growth of young chickens, but did not produce symptoms of chick edema disease. The broad band in chromatogram (a) with a maximum at 8.4 is not characteristic of a toxic fat. No additional work has been done to identify the substances causing these broad bands.

Twelve commercial vegetable oil fatty acids, nontoxic by the chick edema bioassay, were examined. These samples included fatty acids from coconut, cottonseed, corn, palm, soybean, and tall oils. Eleven of the samples were negative by the chromatographic method, but one of the samples (a tall oil fatty acid) gave a small chromatographic peak with $R_A = 5$, indicative of a toxic sample.

**Acknowledgments**

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# Methods of Analysis for Tall Oil Products

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THE TALL OIL INDUSTRY in the United States has grown at a phenomenal rate during the past 10 years. Highly refined products are now being produced from tall oil, such as very light-colored fatty acids, substantially free from rosin acids and rosin substantially free from fatty acids. Manufacturers of paint, varnish, soap, detergents, linoleum, and paper have adopted these products as raw materials and depend on their quality and uniformity. It is therefore not surprising that, in the United States, considerable attention has been given to, and a lot of work has been done on the development of suitable methods of analysis for these products.

Most of this work was carried out in laboratories of producers and users of tall oil products in collaborative tests under the auspices of the American Society for Testing Materials (Committee D-17) with the support of the Tall Oil Division of the Pulp Chemical Association. Some methods developed by committees of the American Oil Chemists' Society for the analysis of fatty acids have also found application for tall oil fatty acids. It is the purpose of this paper to give a short review of some of the A.S.T.M. work and to discuss present methods for the analysis of tall oil products in general.

## Tall Oil and Tall Oil Fatty Acids

Analytical values which are often or occasionally determined on crude tall oil, acid-refined tall oil, distilled tall oil, and tall oil fatty acids are as follows:

*Chemical Values.* Acid number, saponification number, rosin acids, fatty acids, unsaponifiables, polyunsaturated fatty acids (conjugated and total linoleic acid), saturated fatty acids, oleic acid, and iodine value.

*Physical Values.* Color, moisture, specific gravity, refractive index, viscosity, flash point, pour-point, cloud-point, and titer.

Values for typical tall oil products in the United States are given in Table I.

Acid values and saponification values are determined according to the time-honored methods of the oil and fat chemists. Methanol is universally used as a solvent and methanolic KOH as titrant. While potentiometric titration is specified in the referee methods of the A.S.T.M., the use of thymol blue or phenolphthalein as indicator is popular in many laboratories because of speed or convenience.

The determination of rosin acids is more complicated and has a long history of development. The first to solve this problem was Twitchell (1). By bubbling hydrochloric acid gas through solutions of rosin and fatty acids in alcohol, he made use of their different esterification rates and was able to convert the fatty acids into their respective esters while the rosin remained unesterified and could be titrated. This principle of selective esterification is still the basis of all present methods, which in the meantime have undergone many changes to make them simpler and faster. Wolff (2) in 1910 introduced the use of sulfuric acid as an esterification catalyst. There were a number of investigations in Germany, Sweden, and the U.S.A., which contributed to methods of rosin acids determination. While in Europe the slower gravimetric methods, in which the fatty acid esters are isolated and weighed, became standards of the industry; in the U. S. emphasis was placed on simple manipulation and speed. A simple and fast modification of the Wolff method was developed by Hastings and Pollak and published in 1939 (3). It consisted in refluxing a sample of tall oil in methanol in the presence of sulfuric acid for 2 min., cooling, and titrating the unesterified rosin acids by using a thymol blue indicator. This indicator, because of its two color changes, allows measurement of the rosin acid aside from the mineral acid in one titration. The A.S.T.M. adopted this method and added potentiometric titration, which eliminates difficulties in determining the end-points, particularly in such dark-colored products as crude tall oil (4).

The McNicoll method (5), which uses naphthalene sulfonic acid as a catalyst and titrates unesterified rosin acids with phenolphthalein as indicator against a blank was first employed for the determination of rosin in soap. It too became an A.S.T.M. method for tall oil.

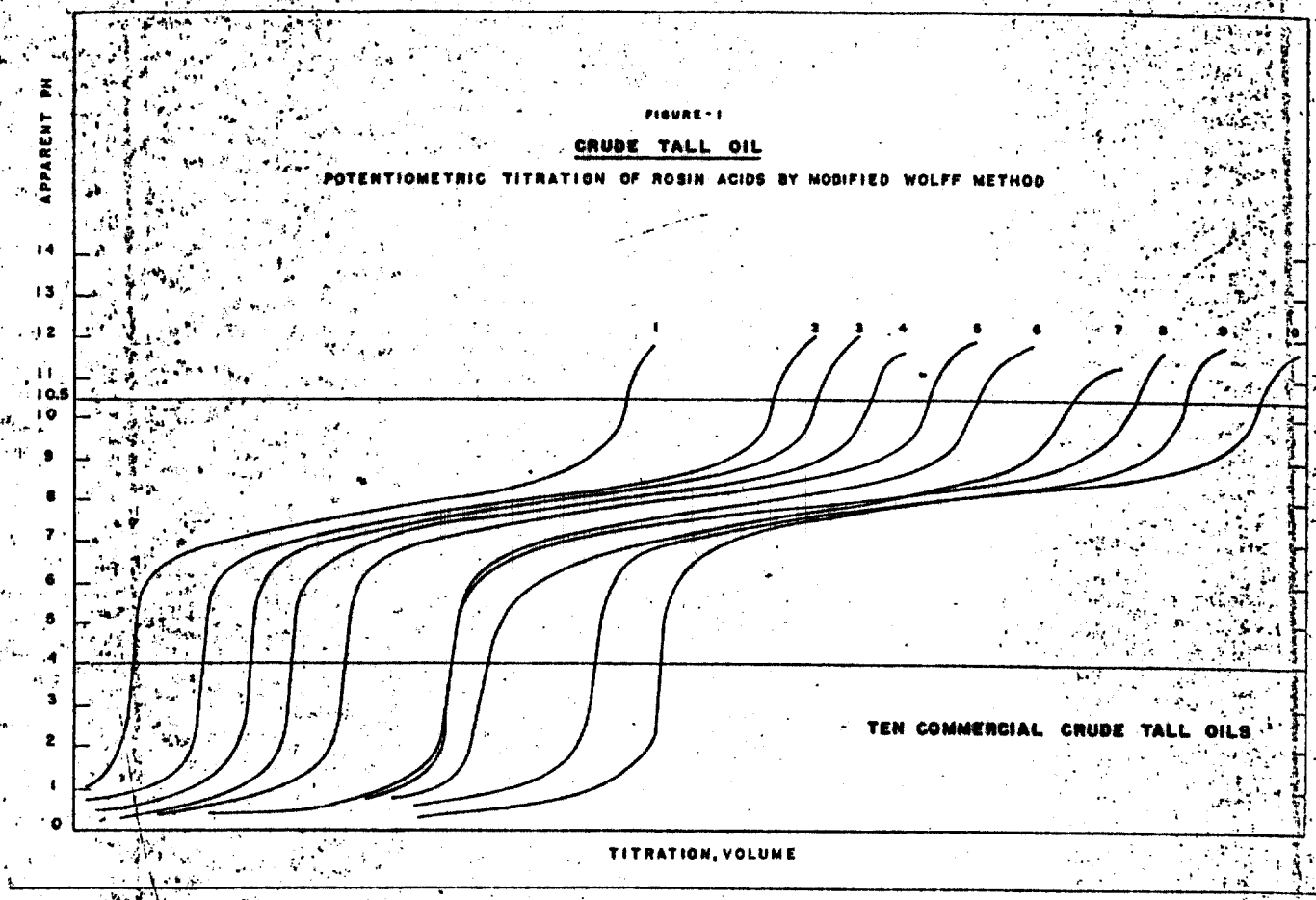
By 1953 experience in our own laboratory and investigations by Sprengling (6) and Hezel (7) indicated that the reflux time of 2 min. for the Wolff Method was too short. We decided to determine the reflux times necessary for rosin acid mixtures of 30 to 50%, corresponding to the crude and refined tall oil products of commerce. The mixtures were prepared from rosin-free distilled soybean fatty acids and from tall oil rosin acids repeatedly recrystallized from methanol. A Beckman Model K automatic titrator was used. To determine the end-points which would give the most accurate results in connection with automatic titrators, potentiometric titration curves were first plotted with 10 different commercial crude tall oils, four distilled tall oils, and two acid-

TABLE I

Analysis Values of Typical U. S. Tall Oil Products

| Product | Acid No. | Sap. No. | Rosin acids | Unsap. | Fatty acids | Color, Gardner | Specific gravity 25°/25°C. | Refractive index 25°C. |
|---|---|---|---|---|---|---|---|---|
| | | | % | % | % | | | |
| Crude tall oil | | | | | | | | |
| Low rosin | 170 | 174 | 40.0 | 6.9 | 53.5 | 17 | 0.960 | 1.5030 |
| High rosin | 166 | 172 | 49.0 | 7.1 | 43.1 | 18 | 0.975 | 1.5100 |
| Distilled tall oil | 190 | 194 | 29.0 | 1.2 | 69.8 | 7+ | 0.947 | 1.4860 |
| Acid refined tall oil | 169 | 173 | 40.0 | 6.8 | 53.1 | 8 | 0.930 | 1.5025 |
| Tall oil fatty acids | 192 | 194 | 4.0 | 4.0 | 92.0 | 7+ | 0.901 | 1.4710 |
| | 196 | 197 | 1.8 | 1.5 | 97.2 | 5+ | 0.900 | 1.4670 |
| | 198 | 199 | 0.4 | 0.4 | 99.2 | 3 | 0.897 | 1.4645 |
| Tall oil heads | 173 | 177 | 0.5 | 15 | 84.4 | 12+ | 0.905 | 1.4754 |
| Tall oil rosin | 168 | 176 | 94[a] | 4.0 | 2.0 | WW[b] | 1.060 | — |
| Tall oil pitch | 55 | 115 | 32 | 32 | 30 | 17[c] | 1.005 | — |

[a] Includes rosin esters and anhydrides. [b] U. S. rosin color standards. [c] 10% solution in benzene.




refined tall oils (Figures 1 and 2). The results show inflection points of pH 4 and 10.5 for either crude tall oil, distilled tall oil, or acid-refined tall oil. With most tall oil products titration to definite end-points is satisfactory, and interpretation of individual titration curves is not necessary. It was found that a 15- to 30-min. esterification time is necessary with mixtures containing between 30 to 50% rosin acids. The tests were repeated and confirmed in a collaborative testing program by members of A.S.T.M. in 1954. As a result of this work the Wolff method was changed to the present A.S.T.M. D 803-55T, which specifies a 30-min. reflux time and allows the use of an automatic titrator with pH 4 and 10.5 end-points as an alternate to plotting a titration curve.

One of the greatest challenges to the tall oil chemist was to provide an accurate enough method for the determination of the small percentages of rosin acids in the highly fractionated tall oil fatty acids produced in modern distillation plants. The Herrlinger-Compeau method (8) solved this problem by using a relatively large sample of fatty acid (40 g.), refluxing it in methanol in the presence of sulfuric acid, cooling, adding ether, extracting the mineral acid with salt solution, and titrating the remaining unreacted rosin acids dissolved in the ether. This method was adopted by the A.S.T.M. for the determination of rosin acids in fatty acids and is now A.S.T.M. D-1240-54.

The testing of this method by the A.S.T.M. in 1951 is one of the best examples of the type of collaborative work carried out by its members. Samples of fatty acids containing 0 to 15% rosin acids were sent to 19 laboratories, including those of producers and users of tall oil products, government laboratories, commercial testing laboratories, and research institutions. With the exception of one laboratory none had previous experience with this method.

TABLE II

Results of Collaborative Test of Herrlinger-Compeau Method

| A.S.T.M. member laboratory | Rosin acids content of samples | | | | | |
|---|---|---|---|---|---|---|
| | 0.00 | 1.00 | 3.00 | 6.00 | 10.00 | 14.00 |
| | Rosin acids found | | | | | |
| 1 | 0.02 | 1.03 | 3.05 | 6.03 | 9.95 | 13.8 |
| 2 | 0.04 | 1.00 | 2.99 | 6.00 | 9.88 | 13.7 |
| 3 | 0.05 | 1.06 | 2.99 | 6.08 | 9.95 | 13.7 |
| 4 | 0.01 | 0.99 | 2.94 | 5.99 | 9.84 | 13.5 |
| 5 | 0.38 | 1.16 | 3.18 | 6.27 | 9.84 | 13.9 |
| 6 | 0.45 | 1.28 | 3.79 | 5.37 | 9.95 | 13.9 |
| 7 | 0.09 | 1.06 | 3.07 | 6.00 | 10.70 | 13.8 |
| 8 | nil | 0.96 | 2.09 | 5.99 | 9.83 | 13.5 |
| 9 | 0.01 | 0.811 | 3.09 | 6.09 | 9.94 | 13.9 |
| 10 | 0.076 | 1.02 | 3.08 | 6.80 | 10.7 | 15.3 |
| 11 | — | 0.81 | 3.08 | 6.03 | 10.15 | 15.0 |
| 12 | 0.00 | 0.93 | 2.97 | 5.98 | 9.94 | 13.8 |
| 13 | 0.145 | 1.08 | 3.15 | 6.16 | 9.76 | 13.5 |
| 14 | 0.21 | 0.99 | 3.07 | 6.06 | 9.83 | 13.5 |
| 15 | 0.25 | 1.16 | 3.30 | 6.23 | 10.12 | 14.0 |
| 16 | −0.04 | 1.29 | 2.94 | 5.81 | 9.75 | 13.2 |
| 17 | 0.02 | 0.98 | 2.97 | 5.98 | 9.88 | 13.8 |
| 18 | 0.02 | 1.06 | 3.05 | 6.06 | 10.06 | 13.9 |
| 19 | −0.14 | 1.00 | 2.93 | 5.92 | 9.94 | 13.6 |

The results of the collaborative tests are given in Table II. They can be summarized as follows:

| | |
|---|---|
| Samples tested | 114 |
| Number of collaborative laboratories | 19 |
| Rosin acids range | 0–15% |

| Determinations | | Accuracy |
|---|---|---|
| Number | Percentage | Rosin acids content |
| 76 | 67 | ±0.1% |
| 19 | 17 | ±0.2% |
| 9 | 8 | ±0.3% |

TABLE III

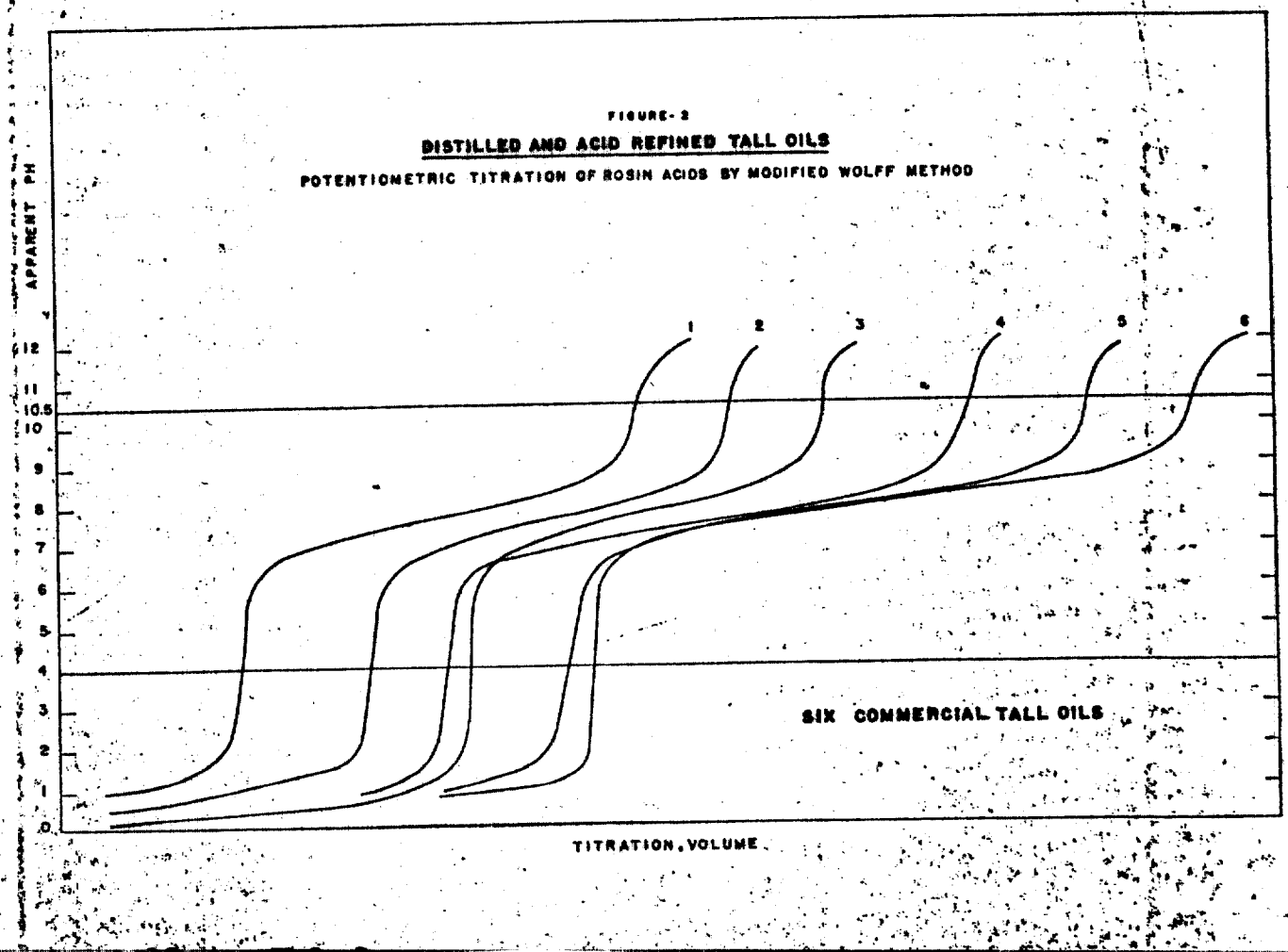
A.S.T.M. Methods for Determination of Rosin Acids

| Method | | Principle of method | Range rosin acids | Application | | |
|---|---|---|---|---|---|---|
| | | | | Tall oil | Fatty acids | Rosin |
| | | | % | | | |
| Wolff | D 803-55T | Methanol, sulfuric acid, 30-min. reflux | 15–55 | X | — | — |
| Wolff[a] | — | Methanol, sulfuric acid, 5-min. reflux | 55–100 | — | — | X |
| Linder Persson[a] | — | Butanol, sulfuric acid, azeotropic removal of water | 0–100 | X | X | X |
| Herrlinger-Compeau | D1240-54 | Methanol, sulfuric acid, 40-g. sample extraction of sulfuric acid before titration | 0–15 | — | X | — |
| McNicoll | D 803-55T | Methanol naphthalein sulfonic acid | 15–55 | X | — | — |

[a] A.S.T.M. designation to be issued.

A new method for the determination of rosin acids applicable over the whole range of rosin content from tall oil fatty acids to tall oil rosin is the revised Linder Persson Method (9). The sample is esterified with butyl alcohol in the presence of benzene and sulfuric acid. The water formed during the esterification is removed by azeotropic distillation and collected in a specially constructed moisture trap. After titration, using either a thymol blue or phenolphthalein indicator, a correction factor is applied to the result. In the U. S. this method is being tried with good results by a few people for tall oil products. It has been tested by the A.S.T.M. in connection with the determination of fatty acids in rosin and found to give results equivalent to the Wolff method. Since it is claimed that the Linder Persson method has the advantage of applicability to products with widely varying rosin content, it will undoubtedly be further investigated by the A.S.T.M. A summary of the A.S.T.M. Methods for rosin acids determination is given in Table III.

The method which is used for the determination of unsaponifiables in tall oil and tall oil fatty acids, A.S.T.M. D 803-55T, has been carefully worked out over the years by the members of A.S.T.M. It consists of refluxing a 5-g. sample in 15 ml. of 2N alcoholic potassium hydroxide for 1.5 hrs., followed by extraction of the unsaponifiables from the soap solution with ether by using conventional separatory funnels. The conditions for dilution, extraction and isolation of the unsaponified matter are critical for obtaining reproducible results. The higher alkali concentration assures complete saponification of the rosin esters present, and the small volume of the alcoholic alkali eliminates the need for alcohol removal prior to extraction. For removal of the soap the ether extract is washed with gradually increasing quantities of water. This prevents the formation of emulsions. After weighing, the unsaponifiables are dissolved in isopropyl alcohol and titrated with alkali to correct for rosin carried over through hydrolysis of the rosin soap. Collaborative work was carried out by A.S.T.M. members from 1952 to 1954 to bring this method to its present form and to adopt it as an alter-



nate method for the analysis of unsaponifiables in rosin.

Moisture is determined by azeotropic distillation of a large sample with xylene and collection of the water in the Dean-Stark Trap. Only crude tall oils contain moisture. Distilled and refined products are dry.

The fatty acid content of crude tall oil, distilled tall oil, acid-refined tall oil, and tall oil fatty acids is calculated from the difference between 100 and the sum of rosin acids, unsaponifiables, and moisture. This is in contrast to the determination of fatty acids in rosin, which is calculated from the acid number and the rosin acids content.

Individual fatty acid constituents are quite often determined to furnish product specification data and to judge the effect of operational variables and seasonal changes in the raw material. These are conjugated, nonconjugated, and total polyunsaturated fatty acids (which are usually expressed as linoleic acid), oleic acid, and saturated acids (mainly palmitic acid). The determinations can be carried out directly on highly refined tall oil fatty acids if they are low in both unsaponifiables and rosin. On products with higher rosin and unsaponifiable content and for most accurate results, the fatty acids must first be isolated. To separate the fatty acids a sample is esterified with methanol and the rosin is extracted with alkali. The ester is saponified, and the unsaponifiables are removed by extraction with ether. The fatty acids are then liberated from the remaining soap solution, taken up in ether, washed acid-free, and dried.

Conjugated and nonconjugated polyunsaturated fatty acids are determined according to the modified Brice and Swain ultraviolet spectrophotometric method A.O.C.S. L12a-55 and Cd 7-48, using a Beckman Model DU quartz spectrophotometer. This method is based on the measurement of conjugated linoleic acid by ultraviolet absorption before and after isomerization of the sample by heating with a glycol or glycerol sodium hydroxide solution, which converts the nonconjugated linoleic acid to the conjugated type. Since the polyunsaturated acids consist mainly of dienoic acids with less than 1% of trienoic acids and no acids of higher unsaturation, readings are taken at wavelengths of 233, 262, 268, and 274 mμ only.

Saturated fatty acids, which in tall oil fatty acids consist mainly of palmitic acid, are determined according to the lead salt method A.O.C.S. Cd 6-38. Since rosin is also precipitated, a correction for the rosin content of the sample must be applied. With certain products the lead salts do not crystallize readily. A method which is sometimes better but is also subject to a lot of complications is the separation of the solid acids by low-temperature crystallization from a methanol-water mixture. The determination of palmitic acid can also be carried out by a quantitative fractional distillation of the methyl esters. None of these methods is very satisfactory. It is expected that vapor chromatographic separation of the methyl esters will not only allow an accurate determination of palmitic acid but also the direct determination of oleic acid, linoleic acid, and higher molecular saturated and unsaturated fatty acids. Oleic acid is, at present, determined as the difference between the total fatty acids and the sum of saturated and polyunsaturated fatty acids.

As with most refined products, color has become an important criterion of quality. It is at present determined with a Hellige color comparator and the Gardner Varnish scale. This has so far been the most satisfactory method even for the extremely pale-colored tall oil fatty acids. The determination of color by using the Lovibond Scale has not found adoption among users and producers of tall oil products. The reason is that the method is cumbersome, and the color standards are expensive to maintain. However there is room for an improved method, particularly for the determination of very light colors where a more discriminating method is needed. Spectrophotometric measurement of color promises to eliminate the human factor of visual determinations. A committee of the A.O.C.S. is working on this problem.

Specific gravity is important for weight-per-gallon information needed for shipping and storage. Viscosity and flash-point are usually given in a typical analysis for customers' information.

Refractive index is one of the most useful values for production control in tall oil refining plants. It takes less than one minute to run a refractive index, yet the value obtained is highly significant in judging quality changes in the finished and intermediate products. The refractive index of a specific product is influenced mainly by the rosin and the unsaponifiables content and to a minor degree by the composition of the fatty acids. While the relationship between refractive index and composition varies from product to product, as well as for the same product made at different times, it can nevertheless be used to determine changes in the rosin and unsaponifiable content of still products by using factors determined from time to time by actual chemical tests. For example, the total of the rosin acids and unsaponifiable content can be determined by refractive index, using the latest factor for the product in question. Then if the rosin acids content is known (*i.e.*, recently determined by chemical analysis) the unsaponifiable content can be calculated by the difference, or, if the unsaponifiable content is known, the rosin acids can be calculated. A typical curve showing the relationship between the refractive index and the total for rosin acids and unsaponifiables during a certain production period is shown in Figure 3.

Pour-point, cloud-point, and titer are often requested by customers who are concerned with the storing qualities of tall oil and tall oil fatty acids. Unfortunately none of these values can be accurately determined in tall oil products, or, if determined, the data are of no value in judging the behavior of the products on storage. There are two constituents in tall oil and tall oil fatty acids which at low temperature can crystallize and settle from solution. One is rosin, which readily forms supersaturated solutions. These may remain liquid for days and then unpredictably set up solid even at room temperature. Neither the pour-point nor the titer would detect supersaturation. The other constituent is palmitic acid. It comes out more readily on cooling, first as a fine cloud and finally settling out as a bottom layer after 24–48 hrs. A titer test which is determined by the heat of crystallization of the solid acid, in this case palmitic acid, will not show up unless the concentration of the solid acid is high enough. Most distilled tall oils and tall oil fatty acids contain less than 3% of palmitic acid, a concentration too low to give a noticeable exotherm on cooling of the sample. The cloud-point will vary and not tell the story either. The most reliable test

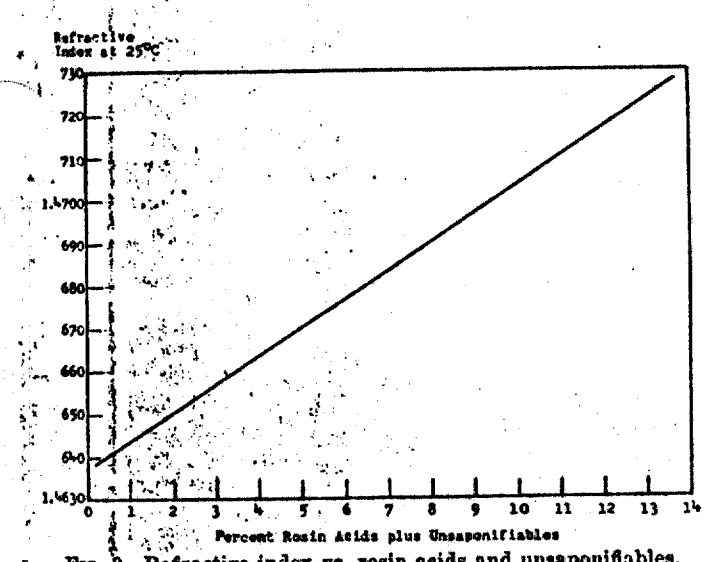



FIG. 3. Refractive index vs. rosin acids and unsaponifiables.

for storage behavior is to place the sample in a refrigerator at the desired temperature for testing, shaking, and examining daily for several days.

Another test which is not suitable for tall oil and tall oil fatty acids is the Iodine Number. While this value is of considerable significance in judging the unsaturated fatty acid content of vegetable and marine oils in connection with their drying qualities, it cannot be used to judge tall oil products because both rosin and unsaponifiables greatly influence the iodine test. A better method for judging the quality of tall oil fatty acids in connection with drying properties is to determine the conjugated and total linoleic acid content by spectrophotometric analysis.

## Tall Oil Rosin

Analytical values usually or occasionally determined on tall oil rosin are the following:

*Chemical Values.* Acid number, saponification number, rosin acids, fatty acids, unsaponifiables, ash, iron, abietic acid, and dehydroabietic acid.

*Physical Values.* Color, hardness, optical rotation, and softening point.

Acid number and saponification number are carried out according to A.S.T.M. D 465-51 and A.S.T.M. D 464-51, respectively. Referee methods use potentiometric titration, and the alternate indicator methods use phenolphthalein as an indicator. Solvents are ethyl or methyl alcohol with the addition of some benzene, to effect a clear solution. The acid number is perhaps the most important criterion for rosin quality. It is a measure of the acid equivalents available for chemical reactions and indicates whether the rosin has been subjected to heat treatment or other modification such as neutralization.

Since tall oil rosin is separated from fatty acids by a fractional distillation process, the determination of residual fatty acids is of great importance for the purpose of plant control during production to assure a high quality product. Producers regulate their processes so as to obtain a clear, hard, vitreous high-melting rosin containing usually less than 3% of fatty acids.

The method used for the determination of fatty acids in rosin had originally been developed in our laboratories. Its principle is to calculate the fatty acids content from the difference between the total acidity of the sample, determined as acid number, and the acidity contributed by the rosin acids, determined either according to a modified Wolff method or the Linder Persson method. Since the fatty acids remaining in tall oil rosin consist of oleic acid and higher molecular fatty acids of varying compositions, it has become customary to compute and report the fatty acids content as oleic acid.

The A.S.T.M. has recently carried out a collaborative testing program on modifications of this method, which are now in the process of being adopted as standards. A short outline of the method is as follows:

a) Determine the acid number of the rosin;

b) determine rosin acids by using either a Wolff method with 5 min. of reflux time or the Linder Persson method;

c) calculate fatty acids content from acid number and rosin acids content as follows:

$$\text{Fatty acid \% (as oleic acid)} = \frac{AN - (\%\,RA \times 1.855)}{1.986}$$

where

AN = acid number

RA = rosin acids

There are two methods for the determination of unsaponifiables in rosin, A.S.T.M. D 1065-56, Methods A and B. Method A is a continuous-extraction method; Method B, a separatory funnel method identical to A.S.T.M. D 803-55T for tall oil. For some time the continuous-extraction method has been the only standard. Many users of rosin and private testing laboratories did not have the continuous-extraction equipment. Tall oil producers who entered the rosin field in 1950 used their funnel method for the determination of unsaponifiables in tall oil. The A.S.T.M. therefore decided to develop an alternate funnel method for rosin which would give values identical to the continuous method. The A.S.T.M. subcommittee on rosin carried out considerable work during 1952–1954 on these methods. It was found that a higher alkali concentration (2N) during the saponification step gave more reliable results in both methods and allowed some simplification in the extraction method. The revised methods were tested on gum rosin, wood rosin, and tall oil rosin. The results obtained by eight analysts of six A.S.T.M. member laboratories with tall oil rosin, comparing the revised continuous-extraction method with the separatory funnel method, Method D 1065-56, Methods A and B, are shown in Table IV.

The method for ash is A.S.T.M. D 1063-51 and for iron, A.S.T.M. D-1064-51. Neither method is used often on tall oil rosin. Since tall oil rosin is made by distillation, it should be very low in both ash and iron unless iron is picked up from the still, which then indicates the existence of a serious corrosion problem for the producer.

TABLE IV

Results of A.S.T.M. Collaborative Tests of Method for Unsaponifiables in Rosin

| | Results with tall oil rosin | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Analyst | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Method A | 3.3 | 3.4 | 3.5 | 3.4 | 3.4 | 3.8 | 3.5 | 3.4 |
| | 3.3 | 3.3 | 3.3 | 3.4 | 3.5 | 3.7 | 3.6 | 3.4 |
| | 3.3 | 3.4 | 3.5 | 3.5 | 3.4 | — | — | — |
| Method B | 3.8 | 3.7 | 3.7 | 3.1 | 3.4 | 3.6 | 2.8 | 3.6 |
| | 3.7 | 3.6 | 3.7 | 3.2 | 3.4 | 3.7 | 3.6 | 3.7 |
| | 3.7 | 3.7 | 3.7 | 3.4 | | | | |

Method A. Separatory funnel method.
Method B. Continuous extraction method.

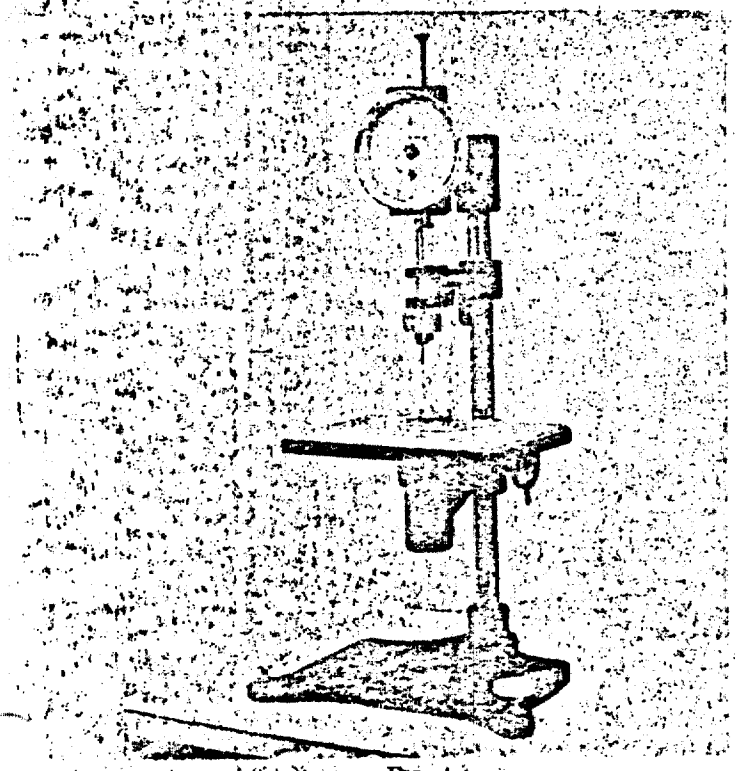


FIG. 4.

Abietic and dehydroabietic acid is determined on tall oil rosin which has been subjected to some treatment such as disproportionation or dehydrogenation to produce rosin specialties. In general, a modification of the method developed during World War II by the Rubber Reserve (10) is used. A sample is dissolved in either ethanol or methanol, and its absorption is measured at wavelengths 241, 273, 276, 279, and 300 mμ, using a Beckman Model DU quartz spectrophotometer.

Color is determined according to A.S.T.M. D 509-55, using U. S. government rosin standards.

The hardness of tall oil rosin is a good criterion for low fatty-acid content. It is not satisfactory for quantitative determinations but can be used with advantage as a quick test for control of still operations. We adopted for this purpose a Precision Universal A.S.T.M. Penetrometer. The rosin is poured into a mold consisting of a ring ¼ in. high and ¾ in. in diameter. The rosin held by the ring is cooled to 25°C. in a water bath, and the penetration is determined by using a penetrometer needle, a 150-g. weight, and a 10-second release time. (The rosin cubes used for color determinations, suitably cooled, may also be used.) We have recommended this method to our friends, and it is now used by several producers of tall oil rosin. The needle must be replaced frequently to assure uniformity of results. While it is a good qualitative method, all efforts to make it a quantitative measure for fatty acid have failed because of the many factors that influence hardness in rosin.

Optical rotation is very useful for the control of processing rosin by heat treating, disproportionation, and dehydrogenation. We dissolve the rosin in chloroform and determine its rotation in a 1-decimeter tube, using a Rudolf Model 60 Polarimeter with a sodium lamp. The results are expressed as specific rotation. The rotation can also be measured on the solid rosin, which is poured into a special metal and glass cuvette and allowed to cool. The results are not as accurate because strains set up in the rosin during cooling influence the rotation.

The softening point of rosin is determined by the ring and ball method A.S.T.M. E 28-51T.

## Summary

The principal standard methods used by the American industry for the analysis and quality control of tall oil products have been discussed, and some of the work of the A.S.T.M. in developing these methods has been reviewed.

Further growth of the tall oil industry will undoubtedly result in new products of greater refinement and wider utilization. New and improved methods of analysis will be required. These can be worked out by the industry in continued collaborative work under the auspices of the A.S.T.M. and A.O.C.S. Undoubtedly instrumental test methods such as infrared and ultraviolet spectroscopy and gas chromatography will play a role of increasing importance in the analysis of tall oil products.

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# Effect of Flotation Reagents on Fish and Animal Fish Feed

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## Introduction and Literature Review

The term "flotation" is used to describe the mechanical separation of mixtures of solid, finely granulated products, which are obtained in an aqueous medium by addition of flotation reagents. The process is based on the different wetting power of the individual components, achieved by the addition of flotation agents.

In general, flotation agents used include collecting, foaming, and regulating agents. The collecting agents (or collectors) are deposited (attached) on the surfaces of specific components and render the latter hydrophobic or water-repellent. When air is blown or mixed in, these particles then become attached or cling to the rising bubbles and reach the turbid surface together with the latter. The task of the foaming agents is to produce a stable foam, with which the suspended solid particles can be removed on the turbid surface. Addition of these reagents is also important in achieving a favorable distribution of air in the turbid mixture. Most collectors do not exert a selective effect on only one mineral product. In order to achieve the removal from the foamed product of only the desired components, definite flotation conditions must in most cases be

established by adding so-called regulators. In addition, reagents must often be added which prevent other components from rising to the surface of the mixture. Such flotation agents are known as suppressors.

Both collectors and foaming agents contain a nonpolar and a polar group in their molecule. The former group is hydrophobic and aerophilic, while the latter group is reactive and can be adsorbed by the particles. It is assumed that minerals are concentrated with the collector only in an extremely thin layer, whereby the polar residue of the molecule is bound to the border surface, whereas the nonpolar, aerophilic group is directed outwards and exerts a hydrophobic effect.

In some respects, flotation reagents and detergents (synthetic washing products) are very closely related. Frequently, it is quite impossible to draw a difference between collectors and foaming agents on the one hand, and washing products on the other. In the same way as by detergents, these flotation reagents can also be divided into anion-active, cation-active, and non-ionic compounds, according to the active component in the molecule of the surface-active compound.

Whereas the toxic effect of detergent-containing effluents on fish and animal fish feed has already been described in numerous publications (for a summary listing, see references 1, 2, and 3), only the often very high and fishery-damaging content of turbid products found in flotation effluents has been discussed in the past (4).

Results of tests aimed specifically at the toxic effect on fish of flotation chemicals are found only in some Austrian publications. In tests with minnows (Phoxinus laevis), Slanina (5) found that no visible injuries were observed in the test animals after 7 days when the concentration of the flotation agents was below the following values: Flotanol 200 mg/l, fusel oil 100 mg/l, potassium amyl xanthogenate 25 mg/l, potassium ethyl xanthogenate 6 mg/l, technical-grade sodium sulfide 6 mg/l, and Tragol 5 mg/l. Slanina believes that already at low concentrations of the flotation effluents there is no direct danger for lower fauna and fish in draining ditches.

Weber (6) studied the toxic action of flotation agents used in the production of magnesite. According to his data, 6,500 l. of water and 3 kg each of Lipol, as collector and foaming agent, and DD95, as "suppressor salt", is added per cubic meter of crude magnesite. The limit of toxicity of Lipol for rainbow trout was between 25 and 50 mg/l, based on a water temperature of 15°C. The DD95 suppressor salt is less toxic and killed trout only at a concentration of 200 mg/l. Whereas Lipol lost practically all of its toxicity as a result of adsorption by the finely ground solid product, the toxicity of the suppressor salt appeared to be only slightly reduced in this manner. Therefore, Lipol does not present an immediate danger to trout, in spite of its toxicity in waters located downstream from magnesite plants. In this particular case, the suppressor salt also would not appear to reach a concentration capable of causing the death of fish living in such waters.

## Experimental Method

In studies performed so far, technical products were mostly used, the composition of which was not reported in detail. Frequently, only the trivial names of flotation agents were given. In collaboration with the Research Institute for Ore Dressing of the German Academy of Sciences in Berlin, the toxicological effect on fish and animal fish feed of a series of flotation reagents was investigated, whereby the composition and concentration of the active ingredient of these reagents was approximately known. (Note: We wish to thank Prof. Dr. Kirchberg for encouraging us to perform this work and for supplying the necessary flotation reagents.)

The following anion-active, cation-active, and non-ionic reagents were studied:

1. Anion-active flotation reagents
    a. Acids: Oleic acid, p-tolyarsonic acid, tall oil (30-40% resin acids [abietic acid], 40-55% fatty acids [oleic, linolic and linolenic acids], 12.5% unsaponifiables [hydrocarbons, phytosterol], 2% petroleum ether-insoluble residue).
    b. Sulfonates: $C_{12}$-alkylphenyl sulfonate (85%, residue: NaCl, $Na_2SO_4$), $C_{10}$-$C_{16}$ alkyl sulfonate (total fat content 33%, sulfonation degree 80%), mersol sulfonate, average chain length $C_{15}$ (95%, residue: 3% NaCl, 1% $Na_2SO_4$, 1% alkanes).

c. Xanthogenates: Potassium ethyl, butyl and isoamyl xanthogenates.

d. Dithiophosphates: Aerofloat 25 (sodium dicresyl dithiophosphate).

2. Cation-active flotation reagents.

a. Pyridinium salts: $C_9$-alkyl pyridinium bromide (36%), $C_{12}$-$_{16}$ alkyl pyridinium bromide (68%), $C_{18}$ alkyl pyridinium bromide (74%).

b. Amines: $C_{10}$-amine hydrochloride (decylamine), $C_{12}$ amine hydrochloride (dodecylamine), $C_{14}$-$_{16}$ amine hydrochloride (23%)base).

3. Non-ionic flotation reagents

a. Terpenes: T-1: terpined (mixture of α- and β-terpineol, 90%; 10% other terpene alcohols and terpene hydrocarbons); T-2: terpineol acetate mixture; T-3: terpene hydrocarbons mixture (main components: dipentene, terpinene, by-products: cineol, eucalyptol, terpineol); T-4: terpene hydrocarbons (Summary formula: $C_{10}H_{16}$); T-5: terpene mixture (mixture of different terpene hydrocarbons such as terpinolene, terpinene, dipentene, further oxidation products of unsaturated terpene hydrocarbons, such as cineole and borneole).

b. Aliphatic hydrocarbons: White spirit (heptane-octane mixture).

The test animals used included perch (Perca fluvialis), roaches (Rutilus rutilus), water fleas (Daphnia spec.), and crayfish (Gammarus spec.).

The fish tested had an average length of 13-18 cm, and were kept, during the 4-day test period, in well-ventilated glass aquariums filled with 10 l. of water. Tests with animal fish feed were performed in flat dishes, filled with 1 l. water and also well-ventilated. Water temperatures ranged from 11 to 17°C, and the tap water used had a pH of 7.8-8.1 and a carbonate hardness of 8° (German hardness scale). Among the flotation reagents used, p-tolylarsonic acid, the sulfonates, xanthogenates, Aerofloat 25, the pyridinium salts and the amines were readily soluble in water. On the other hand, oleic acid, tall oil, all terpenes, and white spirit could only be partly dissolved, so that these compounds had to be used predominantly in the form of aqueous emulsions. The degree of emulsification, achieved by intensive hand shaking, is naturally subject to variations, which again resulted in a certain range of variation of the test results. For this reason, ethanol was experimentally added as a solvent (solution promoter) to tall oil; this alcohol is to a large extent nontoxic for the test animals used.

| (1) Flotationsreagenzien | (2) Barsche (*Perca fluviatilis*) | (3) Schwellenwerte mg/l (4) Plötzen (*Rutilus rutilus*) | (5) Wasserflöhe (*Daphnia spec.*) | (6) Bachflohkrebse (*Gammarus spec.*) |
|---|---|---|---|---|
| Olsäure (7) | > 2000 | > 2000 | — | — |
| p-Tolylarsonsäure | < 1000 (pH) | < 1000 (pH) | 200 | 800 (pH) |
| Tallöl | 10–20 | 20–40 | 40 | 40 |
| $C_{12}$-Alkylphenylsulfonat (8) | 6– 8 | 10 | 30 | 40 |
| $C_{10}$-$C_{16}$-Alkylsulfonat | 12–15 | 50–60 | 10–20 | 150 |
| Mersolat | 6 | 10 | 30 | 30 |
| Kaliumäthylxanthogenat (9) | 2 | 6 | — | > 10 |
| Kaliumbutylxanthogenat | 15 | 20 | — | 50 |
| Kaliumisoamylxanthogenat | 20 | 55 | — | 50 |
| Aerofloat 25 (10) | 50 | 60 | < 50 | 100 |
| $C_9$-Alkylpyridiniumbromid (11) | 160 | 200 | < 5 | 5 |
| $C_{12}$-$C_{16}$-Alkylpyridiniumbromid | 5 | 4 | 0,2 | 1 |
| $C_{18}$-Alkylpyridiniumbromid | 0,75 | 0,75 | < 0,1 | 0,1 |
| $C_{10}$-Aminhydrochlorid (12a) | 2– 3 | 3 | — | 2– 3 |
| $C_{12}$-Aminhydrochlorid | 3– 4 | 4 | — | ~ 4 |
| $C_{14}$–$C_{16}$-Aminhydrochlorid | 4– 5 | 5 | — | 4 |
| T–1 (13) | 25–30 | 35–40 | — | 40 |
| T–2 | 12–15 | 12–15 | — | > 40 |
| T–3 | 30 | 30 | — | > 30 |
| T–4 | 20–30 | 20–30 | 80 | 60 |
| T–5 | 50 | 50 | 125 | — |
| White spirit (14) | 5–10 | 10–15 | 10 | 60 |

Table 1. Toxic action of flotation reagents on fish and animal fish feed.

(1) Flotation reagents

(2) Perch (Perca fluvialis)

(3) Threshold values mg/l

(4) Roach (Rutilus rutilus)

(5) Water flea (Daphnia spec.)

(6) Stream flea crayfish (Gammarus spec.)

(7) Oleic acid
p-Tolylarsonic acid
Tall oil

(8) $C_{12}$-alkyl phenyl sulfonate
$C_{10}$-$C_{16}$-alkyl sulfonate
Mersolate

(9) Potassium ethyl xanthogenate
Potassium butyl xanthogenate
Potassium isoamyl xanthogenate

(10) Aerofloat 25

(11) $C_9$-aklyl pyridinium bromide
$C_{12}$-$C_{16}$-alkyl pyridinium bromide
$C_{18}$-alkyl pydridinium bromide

(12) $C_{10}$-amine hydrochloride
$C_{12}$-amine hydrochloride
$C_{14}$-$C_{16}$-amine hydrochloride

(13) T-1
T-2
T-3
T-4
T-5

(14) White spirit

Tall oil, terpenes, and white spirit had a pleasant aromatic odor, which was characteristic for every compound. The Aeroflat 25 solution had a definite hydrogen sulfide odor, while the $C_{12}$-$C_{16}$-alkyl pyridinium bromide had an intensive pyridine odor. The two other alkyl pyridines, as well as the alkylamines, had a paraffin-like soapy odor, which increased significantly with the length of the alkyl groups. The xanthogenates had a partial stinging and unpleasant odor, apparently due to decomposition products formed from these relatively unstable compounds.

The so-called threshold value was defined by Bandt (7) as a measure of the toxicity of a fish poison. This value is equal to the concentration of toxic product at which the first clearly detectable poisoning symptoms appear in an aquarium test within 3 to 4 days. For example, one of the clearly visible symptoms of poisoning in fish is the appearance of the side (lateral) position.

Some flotation reagents tested led to typical poisoning symptoms. Thus, sulfonates caused extensive mucus secretion in fish. This symptom is also caused by numerous other surface-active products (8). It must be assumed that, in addition to the skin of the fish, also the gill epithelium is destroyed by these compounds, thus resulting in a blocking of gas exchange, and the fish suffocate in spite of a sufficient oxygen content in the water (8).

The terpenes also caused characteristic poisoning symptoms. The fish were at first very quickly stunned and assumed a side position. Frequently, however, they again recovered fully, without requiring a

change of the water in the test tank. The time needed for a complete recovery of roaches may amount to up to 3 days.

This phenomenon is probably due to a partial evaporation of the ethereal (essential) oils, which is further promoted by the introduction of air. Similar observations have also been made by Ebeling (9). Threshold values of a larger number of flotation reagents for fish and animal fish feed are listed in Table 1.

## Discussion of Test Results

From a fishery standpoint, the toxic action of a product can be characterized as follows, according to the range of toxicity limits:

| | |
|---|---|
| below 1 mg/l: | highly toxic |
| 1-10 mg/l: | strongly toxic |
| 10-100 mg/l: | moderately toxic |
| 100-1000 mg/l: | weakly toxic |
| over 1000 mg/l: | practically nontoxic |

Based on this classification, most of the flotation reagents studied must be considered as moderately toxic (threshold values of 10 to 100 mg/l). These include sulfonates, xanthogenates (except for ethyl xanthogenate), all terpenes, further tall oil, Aerofloat 25, and white spirit. As strongly toxic compounds (threshold values 1-10 mg/l), we must consider the amines, and also ethyl xanthogenate and the $C_{12}$-$C_{16}$-alkyl pyridinium bromide. Whereas the $C_{18}$-alkyl pyridinium bromide is an extremely effective toxic product (threshold value <1 mg/l), the $C_9$-alkyl pyridinium bromide must be considered as a weakly toxic compound. Practically nontoxic, from a fishery standpoint,

is only oleic acid and p-tolylarsonic acid. The latter has a strongly acid character, which accounts for the damage suffered by test animals at higher concentrations. At a concentration of 800-1000 mg/l, the lethal pH value for fish in the acid range (11) was reached under our test conditions. The amount of p-tolylarsonic acid required for this purpose is naturally dependent on the buffering degree of the water used.

Upon closer examination of the test results, a particularly striking feature of the alkyl pyridinium salts is the fact that the toxic effect increases greatly with increasing number of C-atoms in the alkyl group. When the amount of sample weighed in is the same, the total number of molecules per liter decreases, however, with increasing molecular weight. As a result, the toxic effect of homologs decreases with an increase of their molecular weight, as was confirmed in tests using xanthogenates and amines, However, since the opposite takes place with the quaternary alkyl pyridinium salts, the toxic effect of these compounds appears to be considerably increased by a lengthening of the alkyl side chain. In this case, little is changed by the fact that the concentration of the active agent in the $C_9$-alkyl pyridinium bromide compound is only about half as great as that of the $C_{18}$-alkyl pyridinium (the content of active agent was measured by determination of pyridine nitrogen by the Kjeldahl method). In regard to their toxic effect, the two products differ, however, by several decimal potencies.

When comparable results are available in the literature, they are in good agreement with those obtained in the present study. Thus,

Leclerc and Devlaminck (12) found that when minnows were exposed for 6 hours, the lethal limit concentration of N- (lauryl-, colamin-, formyl-, or methyl-) pyridinium chloride was equal to 19-20 mg/1. Under the same conditions, the lethal limit concentration of dodecyl benzene sulfonate was 6-7 mg/1. According to the studies of Sierp and Thiele (8), alkylaryl sulfonate kills fish at a concentration of 10 mg/1. Some results are already available on the toxic effect of xanthogenates. According to Ellis (13), gold fish die within 48-96 hours when exposed to potassium xanthogenate (ethyl xanthogenate) concentrations of 10 mg/1. As was already mentioned in the introduction, studies by Slanina (5) show that potassium ethyl xanthogenate and potassium isoamyl xanthogenate no longer injure minnows when their respective concentrations are below 6 and 25 mg/1.

The toxic effect of tall oil is certainly due only to its considerable content of resin acids. Ebeling (9) and Danneel (14) have investigated the toxic effect of resinous products in connection with other matters. These compounds act as nerve poisons, whereby common spruce resin, resin acids (abietic acid) and rosin are about equally toxic for fish. Ebeling states that resins in a concentration of 5 mg/1 kill trout, while causing only a certain amount of injuries to tenches and roaches. According to the same author, gammarids are also injured by resin acids, although these test animals are less sensitive to such compounds, in agreement with our own results. At a resin acid concentration of 20 mg/1, all gammarids were dead after 5 days.

Ebeling (9) also performed tests on fish with two terpene hydrocarbons. The lethal concentration of these compounds for trout was about 5 mg/1, while roaches and bleaks were not yet injured by this amount of toxic product. No information is given on the behavior of the latter two fish species at higher concentrations of these toxic products.

No comparable results were available to us on the toxic effect of the other flotation reagents.

According to Kirchberg (15), the maximum content of reagents in liquid flotation effluents is equal to 20-250 mg/1. The threshold values of most flotation reagents listed in Table 1, however, are considerably lower than 250 mg/1, so that an endangering of fishery draining ditches by such effluents appears quite probable.

There are no specific studies dealing with the adsorption of flotation reagents by minerals. However, even if the major amount of the added chemicals is bound by solid particles (6) and does not reach the draining ditch with the liquid effluents, some of the tested flotation reagents are so highly toxic that even in trace amounts they can poison fish and particularly animal fish feed.

Further, an appraisal of liquid flotation effluents must also take into account the content of regulators in such effluents. A special investigation of this problem could not be carried out in the present study.

Along with other flotation reagents, petroleum or diesel oil are occasionally used as collectors in flotation ore dressing. It is known that both of these products can exert a damaging effect on all

biological relations present in a body of water. According to Bandt (7), the threshold value of diesel oil for roaches is 25-30 mg/l. However, considerably lower concentrations of this product already exert an injurious effect on lower flora and fauna; fish are thereby deprived of their fasic food and thus forced to migrate.

In summary, we can state that the tests performed by us give an overview of the direct toxic effect exerted by a wide variety of flotation reagents. An exact statement as to the extent of the injurious effect exerted by liquid flotation effluents on fishery draining ditches can only be made after determination of the precise content of flotation chemicals in these effluents. Some of the compounds tested, however, are extremely toxic to fish and animal fish feed. It is feared, therefore, that, even after partial adsorption by solid particles, residue of flotation agents remaining in the effluents represents a danger of damage to fishery draining ditches.

## Summary

The mechanical separation of products by the flotation method is recently assuming an increasing importance in the chemical industry. The liquid flotation effluents released in this process contain, along with fine turbid products injurious to fisheries, also certain amounts of added flotation chemicals. For this reason, the toxicological action of a number of flotation reagents on fish and animal fish feed was investigated. Tests were carried out with various xanthogenates, alkyl amines, terpene hydrocarbons, alkyl pyridinium bromides, and alkyl sulfonates. Further, oleic acid, p-tolylarsonic acid, Aerofloat 25, white spirit, and tall oil were also tested. The animals

used in these tests included roaches (Rutilus rutilus), perch (Perca fluvialis), crayfish (Gammarus spec.), and water fleas (Daphnia spec.). The toxic effect of the tested flotation chemicals was in part quite considerable, so that flotation effluents can cause damage to fishery draining ditches both as a result of their content action of fine turbid products and, in some cases, of their toxic action.

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DIETWART NEHRING

# Die Einwirkung von Flotationsreagenzien auf Fische und Fischnährtiere

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## Einleitung und Literaturübersicht

Unter dem Begriff Flotation ist die Trennung von festen, feinkörnigen Stoffgemischen auf mechanischem Wege zu verstehen, die im wässrigen Medium unter Zusatz von Flotationsreagenzien erreicht werden kann. Das Verfahren beruht auf der unterschiedlichen Benetzbarkeit der einzelnen Bestandteile, die durch die Zugabe von Flotationsmitteln bewirkt wird.

An Flotationsmitteln werden im allgemeinen Sammler, Schäumer und Regler zugesetzt. Die Sammler sollen sich an den Oberflächen bestimmter Komponenten anlagern und sie hydrophob, also wasserabweisend, machen. Bei Einblasen oder Einschlagen von Luft können dann diese Teilchen an den aufsteigenden Blasen haften und mit ihnen zur Trübeoberfläche gelangen. Aufgabe der Schäumer ist es, einen stabilen Schaum zu erzeugen, mit dem die aufgeschwommenen Feststoffteilchen auf der Trübeoberfläche abgezogen werden können. Der Zusatz dieser Reagenzien ist auch für eine günstige Verteilung der Luft in der Trübe wichtig. Die meisten Sammler wirken nicht selektiv auf nur ein Mineral. Um zu erreichen, daß allein die gewünschten Komponenten im Schaumgut ausgebracht werden, müssen meist bestimmte Flotationsbedingungen durch die Zugabe sogenannter Regler eingehalten werden. Nicht selten sind außerdem Reagenzien zuzusetzen, welche das Aufschwimmen der anderen Komponenten verhindern. Diese Flotationsmittel werden als Drücker bezeichnet.

Sowohl die Sammler als auch die Schaummittel besitzen in ihrem Molekül eine nichtpolare und eine polare Gruppe. Die erstgenannte Gruppe ist hydrophob und aerophil; die zweite Gruppe ist dagegen reaktionsfähig und kann von den Teilchen adsorbiert werden. Es wird angenommen, daß sich die Mineralien nur in äußerst dünner Schicht mit dem Sammler anreichern, wobei der polare Rest des Moleküls an die Grenzfläche gebunden wird, während die unpolare, hydrophile Gruppe nach außen gekehrt ist und hydrophob wirkt.

Zwischen Flotationsreagenzien und Detergentien* bestehen teilweise sehr enge Beziehungen. Häufig ist eine Unterscheidung zwischen Sammlern und Schäumern einerseits und waschaktiven Substanzen andererseits gar nicht möglich. Ebenso wie bei den Detergentien unterscheidet man auch bei diesen Flotationsreagenzien nach anionaktiven, kationaktiven und nichtionogenen

* synthetisch hergestellte waschaktive Substanzen.

Verbindungen, entsprechend dem wirksamen Bestandteil im Molekül der grenzflächenaktiven Verbindung.

Während über die Schadwirkung von detergentienhaltigen Abwässern auf Fische und Fischnährtiere bereits zahlreiche Veröffentlichungen vorliegen (zusammenfassende Darstellungen vgl. 1, 2, 3) wurde bei den Flotationsabwässern in der Vergangenheit nur deren oft sehr hoher und fischereischädigender Gehalt an Trübstoffen diskutiert (4).

Lediglich von österreichischer Seite liegen einige Versuchsergebnisse speziell über die Giftwirkung von Flotationschemikalien gegenüber Fischen vor. Bei Testversuchen mit Elritzen *(Phoxinus laevis)* fand SLANINA (5), daß nach siebentägiger Versuchsdauer keine sichtbare Schädigung der Versuchstiere eintrat, wenn die Konzentration der Flotationsmittel unter folgenden Werten lag: Flotanol 200 mg/l, Fuselöl 100 mg/l, Kaliumamylxanthogenat 25 mg/l, Kaliumäthylxanthogenat 6 mg/l, Natriumsulfid (techn.) 6 mg/l und Tragol 5 mg/l. SLANINA vermutet, daß bereits bei geringen Verdünnungen der Flotationsabwässer keine unmittelbare Gefahr mehr für niedere Fauna und Fische im Vorfluter besteht.

WEBER (6) untersuchte die toxische Wirkung von Flotationsmitteln, die bei der Gewinnung von Magnesit verwendet werden. Nach seinen Angaben rechnet man pro m³ Rohmagnesit mit einem Zusatz von 6500 l Wasser und je 3 kg Lipol als Sammler-Schäumer und DD 95 als „Drückersalz". Die Schädlichkeitsgrenze von Lipol lag für Regenbogenforellen zwischen 25 und 50 mg/l bezogen auf eine Wassertemperatur von 15 °C. Das Drückersalz DD 95 ist weniger toxisch und tötete Forellen erst bei Konzentrationen von 200 mg/l. Während Lipol durch den feinvermahlenen Feststoff praktisch vollständig entgiftet wurde (Adsorptionswirkung), scheint die Toxizität des Drückersalzes dadurch nur im geringen Maße abgeschwächt zu werden. Das Lipol bedeutet also trotz seiner Giftigkeit in Gewässern unterhalb von Magnesitwerken keine unmittelbare Gefahr für Forellen. Auch das Drückersalz dürfte im vorliegenden Falle kaum eine derartige Konzentration erreichen, die den Tod der darin lebenden Fische verursachen könnte.

## Versuchsdurchführung

Bei den bisherigen Untersuchungen handelte es sich meistens um technische Produkte, über deren Zusammensetzung wenig gesagt wurde. Häufig wurden nur die Trivialnamen der Flotationsmittel genannt. In Zusammenwirken mit dem Forschungsinstitut für Aufbereitung der Deutschen Akademie der Wissenschaften zu Berlin* wurde nun die toxikologische Wirkung einer Reihe von Flotationsreagenzien, deren Zusammensetzung bzw. Wirkstoffkonzentration annähernd bekannt war, auf Fische und Fischnährtiere untersucht.

Zur Untersuchung gelangten anionaktive, kationaktive und nichtionogene Reagenzien:

1. Anionaktive Flotationsreagenzien

a) Säuren:

Ölsäure
p-Tolylarsonsäure
Tallöl [30–40% Harzsäuren (Abietinsäure), 40–55% Fettsäuren (Öl-, Linol- und Linolensäure), 12,5% Unverseifbares (Kohlenwasserstoffe, Phytosterin), 2% Petrolätherunlösliches]

* Herrn Prof. Dr. KIRCHBERG möchten wir auch an dieser Stelle für die Anregung zu dieser Arbeit und für die Bereitstellung der Flotationsreagenzien bestens danken.



b) Sulfonate:

$C_{12}$-Alkylphenylsulfonat (85%ig, Rest: NaCl, $Na_2SO_4$)
$C_{10}$-$C_{16}$-Alkylsulfonat (Gesamtfettgehalt 33%, Sulfonierungsgrad 80%)
Mersolsulfonat, mittlere Kettenlänge $C_{15}$ (95%ig, Rest: 3% NaCl, 1% $Na_2SO_4$, 1% Alkane)

c) Xanthogenate:

Kaliumäthylxanthogenat
Kaliumbutylxanthogenat
Kaliumisoamylxanthogenat

d) Dithiophosphate:

Aerofloat 25 (Natriumdikresyldithiophosphat)

2. Kationaktive Flotationsreagenzien

a) Pyridiniumsalze:

| | |
|---|---|
| $C_9$-Alkylpyridiniumbromid | (36%ig) |
| $C_{12}$-$C_{16}$-Alkylpyridiniumbromid | (68%ig) |
| $C_{18}$-Alkylpyridiniumbromid | (74%ig) |

b) Amine:

$C_{10}$-Aminhydrochlorid (Decylamin)
$C_{12}$-Aminhydrochlorid (Dodecylamin)
$C_{14}$-$C_{16}$-Aminhydrochlorid (23% Base)

3. Nichtionogene Flotationsreagenzien

a) Terpene:

T–1: Terpineol (Mischung von $\alpha$- und $\beta$-Terpineol; 90%ig; 10% andere Terpenalkohole und Terpenkohlenwasserstoffe)
T–2: Terpineolazetatgemisch
T–3: Terpenkohlenwasserstoffgemisch (Hauptbestandteile: Dipenten, Terpinen; Nebenbestandteile: Cineol, Eukalyptol, Terpinenol)
T–4: Terpenkohlenwasserstoffe (Summenformel: $C_{10}H_{16}$)
T–5: Terpengemisch (Gemisch verschiedener Terpenkohlenwasserstoffe wie Terpinolen, Terpinen, Dipenten; ferner Oxydationsprodukte der ungesättigten Terpenkohlenwasserstoffe wie Cineol und und Borneol)

b) Aliphatische Kohlenwasserstoffe:

White spirit (Heptan-Oktan-Gemisch)

Als Versuchstiere wurden Barsche *(Perca fluviatilis)*, Plötzen *(Rutilus rutilus)*, Wasserflöhe *(Daphnia spec.)* und Bachflohkrebse *(Gammarus spec.)* verwendet. Die Versuchsfische waren durchschnittlich 13–18 cm lang. Sie wurden während des 4 Tage dauernden Versuches in gut belüfteten, 10 l Wasser fassenden Glasaquarien gehalten. Die Versuche mit Fischnährtieren wurden in flachen Schalen durchgeführt, die mit 1 l Wasser gefüllt waren und ebenfalls belüftet wurden. Die Wassertemperaturen lagen zwischen 11 und 17 °C, das verwendete Leitungswasser hatte einen pH-Wert von 7,6 bis 8,1 und eine Karbonathärte von 8° d. H. Von den verwendeten Flotationsreagenzien waren die p-Tolylarsonsäure, die Sulfonate, die Xanthogenate, das Aerofloat 25, die Pyridiniumsalze und die Amine im Wasser gut löslich. Dagegen konnten die Ölsäure, das Tallöl sowie sämtliche Terpene und der White spirit nur teilweise in Lösung gebracht werden, so daß diese Verbindungen überwiegend in Form

ihrer wässrigen Emulsionen verwendet werden mußten. Der Emulsionsgrad, der durch intensives Umschütteln von Hand erzielt werden kann, ist naturgemäß Schwankungen unterworfen, was wiederum eine gewisse Schwankungsbreite der Versuchsresultate zur Folge hat. Deshalb wurde beim Tallöl versuchsweise Äthanol als Lösungsvermittler zugesetzt. Dieser Alkohol ist für die verwendeten Versuchstiere weitgehend ungiftig.

Tabelle 1

Giftwirkung von Flotationsreagenzien auf Fische und Fischnährtiere

| Flotationsreagenzien | Schwellenwerte mg/l | | | |
|---|---|---|---|---|
| | Barsche *(Perca fluviatilis)* | Plötzen *(Rutilus rutilus)* | Wasserflöhe *(Daphnia spec.)* | Bachflohkrebse *(Gammarus spec.)* |
| Olsäure | > 2000 | > 2000 | – | – |
| p-Tolylarsonsäure | < 1000 (pH) | < 1000 (pH) | 200 | 800 (pH) |
| Tallöl | 10–20 | 20–40 | 40 | 40 |
| $C_{12}$-Alkylphenylsulfonat | 6– 8 | 10 | 30 | 40 |
| $C_{10}$-$C_{16}$-Alkylsulfonat | 12–15 | 50–60 | 10–20 | 150 |
| Mersolat | 6 | 10 | 30 | 30 |
| Kaliumäthylxanthogenat | 2 | 6 | – | > 10 |
| Kaliumbutylxanthogenat | 15 | 20 | – | 50 |
| Kaliumisoamylxanthogenat | 20 | 55 | – | 50 |
| Aerofloat 25 | 50 | 60 | < 50 | 100 |
| $C_9$-Alkylpyridiniumbromid | 160 | 200 | < 5 | 5 |
| $C_{12}$-$C_{16}$-Alkylpyridiniumbromid | 5 | 4 | 0,2 | 1 |
| $C_{18}$-Alkylpyridiniumbromid | 0,75 | 0,75 | < 0,1 | 0,1 |
| $C_{10}$-Aminhydrochlorid | 2– 3 | 3 | – | 2– 3 |
| $C_{12}$-Aminhydrochlorid | 3– 4 | 4 | – | ~ 4 |
| $C_{14}$–$C_{16}$-Aminhydrochlorid | 4– 5 | 5 | – | 4 |
| T–1 | 25–30 | 35–40 | – | 40 |
| T–2 | 12–15 | 12–15 | – | > 40 |
| T–3 | 30 | 30 | – | > 30 |
| T–4 | 20–30 | 20–30 | 80 | 60 |
| T–5 | 50 | 50 | 125 | – |
| White spirit | 5–10 | 10–15 | 10 | 60 |

Das Tallöl, die Terpene und der White spirit zeichneten sich durch einen angenehm-aromatischen Geruch aus, der für jede Verbindung charakteristisch war. Die Lösung mit Aerofloat 25 roch deutlich nach Schwefelwasserstoff, während dem $C_{12}$-$C_{16}$-Alkylpyridiniumbromid ein intensiver Pyridingeruch anhaftete. Die beiden anderen Alkylpyridine besitzen ebenso wie die Alkylamine einen paraffinartigen, seifigen Geruch, der insbesondere mit der Kettenlänge der Alkylgruppen zunahm. Die Xanthogenate rochen teilweise stechend-unangenehm, was vermutlich auf entstandene Zersetzungsprodukte dieser verhältnismäßig unbeständigen Verbindungen zurückzuführen ist.

Als Maß für die Toxizität eines Fischgiftes wurde von BANDT (7) der sogenannte Schwellenwert definiert. Es ist dies diejenige Giftkonzentration, bei der im Aquarienversuch innerhalb von 3 bis 4 Tagen die ersten deutlich wahrnehmbaren Vergiftungssymptome auftreten. Zu den gut erkennbaren Vergiftungssymptomen gehört bei den Fischen beispielsweise der Eintritt der Seitenlage.



Einige der untersuchten Flotationsreagenzien führten zu typischen Vergiftungserscheinungen. So bewirkten die Sulfonate bei den Fischen eine starke Schleimabsonderung. Diese Erscheinung wird auch durch zahlreiche andere grenzflächenaktive Substanzen verursacht (8). Es muß angenommen werden, daß durch diese Verbindungen neben der Haut der Fische auch die Kiemenepithel zerstört wird. Hierdurch kommt es zu einer Blockierung des Gasaustausches und die Fische ersticken trotz ausreichenden Sauerstoffgehalts im Wasser (8).

Auch die Terpene riefen charakteristische Vergiftungssymptome hervor. Die Fische waren zunächst sehr schnell betäubt und gingen in die Seitenlage über. Häufig erholten sie sich jedoch wieder vollständig, ohne daß ein Wasserwechsel im Versuchsgefäß vorgenommen wurde. Der Zeitraum bis zur völligen Wiederherstellung kann bei den Plötzen bis zu 3 Tagen betragen.

Diese Erscheinung ist wahrscheinlich auf eine teilweise Verflüchtigung der ätherischen Öle zurückzuführen, die durch das Einleiten von Luft noch begünstigt wird. Ähnliche Beobachtungen wurden auch von EBELING (9) gemacht.

In der Tabelle 1 sind die Schwellenwerte für Fische und Fischnährtiere einer ganzen Reihe von Flotationsreagenzien aufgeführt.

## Diskussion der Versuchsergebnisse

Die Giftwirkung einer Substanz kann in fischereilicher Hinsicht entsprechend der Höhe der Toxizitätsgrenze folgendermaßen charakterisiert werden (10):

| | | |
|---|---|---|
| unter | 1 mg/l | hochgiftig |
| 1– | 10 mg/l | stark giftig |
| 10– | 100 mg/l | mäßig giftig |
| 100–1000 mg/l | | schwach giftig und |
| über | 1000 mg/l | praktisch ungiftig |

Die meisten untersuchten Flotationsreagenzien sind auf Grund dieser Einteilung als mäßig giftig zu bezeichnen (Schwellenwerte 10–100 mg/l). Hierzu gehören die Sulfonate, die Xanthogenate mit Ausnahme des Äthylxanthogenats und alle Terpene, ferner Tallöl, Aerofloat 25 und White spirit. Zu den stark giftigen Verbindungen (Schwellenwerte 1–10 mg/l) müssen die Amine gerechnet werden, ebenso wie das Äthylxanthogenat und das $C_{12}$-$C_{16}$-Alkylpyridiniumbromid. – Während das $C_{18}$-Alkylpyridiniumbromid zu den äußerst wirksamen Giftstoffen gehört (Schwellenwert < 1 mg/l), kann das $C_9$-Alkylpyridiniumbromid zu den schwach giftigen Verbindungen gerechnet werden. Praktisch ungiftig ist in fischereilicher Hinsicht nur die Ölsäure und die p-Tolylarsonsäure. Die letzte Verbindung besitzt stark sauren Charakter, worauf auch die Schädigung der Versuchstiere bei höheren Konzentrationen zurückzuführen sein dürfte. Bei etwa 800–1000 mg/l wurde unter unseren Versuchsbedingungen der für Fische tödliche pH-Wert im sauren Bereich erreicht (11). Die hierfür erforderliche Menge an p-Tolylarsonsäure ist selbstverständlich vom Pufferungsgrad des verwendeten Wassers abhängig.

Bei näherer Betrachtung der Versuchsergebnisse ist bei den Alkylpyridiniumsalzen besonders auffällig, daß die Giftwirkung mit steigender Anzahl von C-Atomen in der Alkylgruppe stark zunimmt. Bei gleicher Einwaage verringert sich jedoch die Gesamtzahl der Moleküle pro Liter mit steigendem

Molekulargewicht. Demzufolge nimmt im allgemeinen die Giftwirkung von Homologen mit Ansteigen ihres Molekulargewichtes ab, wie auch die Versuche mit den Xanthogenaten und Aminen bestätigen. Da jedoch bei den quartenären Alkylpyridinumsalzen der entgegengesetzte Fall eintritt, scheint die Giftwirkung dieser Verbindungen durch eine Verlängerung der Alkylseitenkette beträchtlich verstärkt zu werden. Hieran ändert auch die Tatsache wenig, daß die Wirkstoffkonzentration im $C_{12}$-Alkylpyridiniumbromid-Präparat nur etwa halb so groß ist wie diejenige des $C_{16}$-Alkylpyridiniumbromids (der Wirkstoffgehalt wurde durch Bestimmung des Pyridinstickstoffs nach der Kjeldahl-Methode ermittelt). Bezüglich ihrer Giftwirkung unterscheiden sich beide Präparate jedoch um mehrere Zehnerpotenzen.

Soweit vergleichbare Resultate in der Literatur vorliegen, stimmen diese recht gut mit den in der vorliegenden Arbeit erzielten Versuchsergebnissen überein. So fanden LECLERC und DEVLAMINCK (12), daß bei sechsstündiger Einwirkungszeit auf Elritzen die tödliche Grenzkonzentration von N-(laurylcolamin-, formyl- oder methyl-) Pyridiniumchlorid bei 19–20 mg l liegt. Unter gleichen Bedingungen beträgt die Tödlichkeitsgrenze für Dodecylbenzolsulfonat 6–7 mg l. Nach Untersuchungen von SIERP und THIELE (8) wirken 10 mg l Alkylarylsulfonat auf Fische tödlich. Auch über die Giftwirkung der Xanthogenate liegen bereits einige Resultate vor. Nach Angaben von ELLIS (13) sterben Goldfische bei Kaliumxanthogenatkonzentrationen (Äthylxanthogenat) von 10 mg l in 48–96 Stunden. Wie bereits einleitend erwähnt, wirken nach Untersuchungen von SLANINA (5) Kaliumäthylxanthogenat und Kaliumisoamylxanthogenat auf Elritzen nicht mehr schädigend, wenn ihre Konzentrationen kleiner als 6 bzw. 25 mg/l sind.

Die Giftwirkung des Tallöls ist sicherlich allein auf den beträchtlichen Gehalt an Harzsäuren zurückzuführen. EBELING (9) und DANNEEL (14) untersuchten in anderem Zusammenhang die Giftwirkung von Harzstoffen. Diese Verbindungen wirken als Nervengifte, wobei Fichtenharz, Harzsäuren (Abietinsäure) und Kolophonium für Fische etwa gleichermaßen schädlich sind. EBELING gibt an, daß durch 5 mg Harze l Forellen getötet werden, während bei Schleien und Plötzen nur eine gewisse Schädigung eintritt. Gammariden werden nach Angaben des gleichen Autors durch Harzsäuren ebenfalls geschädigt, wenngleich diese Versuchstiere in Übereinstimmung mit eigenen Resultaten weniger empfindlich sind. Bei einer Konzentration von 20 mg l waren nach 5 Tagen alle Gammariden abgestorben.

EBELING (9) führte auch Fischversuche mit zwei Terpenkohlenwasserstoffen durch. Für Forellen lag die tödliche Konzentration dieser Verbindungen bei etwa 5 mg l. Plötzen und Ukelei wurden durch diese Giftmenge noch nicht geschädigt. Über das Verhalten der beiden letzten Versuchsfischarten bei höheren Giftkonzentrationen werden keine Angaben gemacht.

Über die Giftwirkung der anderen Flotationsreagenzien lagen uns keine vergleichbaren Resultate vor.

Nach Mitteilung von KIRCHBERG (15) kann der Gehalt an Reagenzien in Flotationsabwässern maximal 20–250 mg l betragen. Die Schwellenwerte der meisten in Tabelle 1 angeführten Flotationsreagenzien sind aber erheblich kleiner als 250 mg l, so daß eine Gefährdung fischereilich genutzter Vorfluter durch derartige Abwässer durchaus gegeben scheint.

Über die Adsorption von Flotationsreagenzien an Mineralien liegen keine eigenen Untersuchungen vor. Aber selbst wenn die Hauptmenge der zugesetzten Chemikalien durch die Feststoffteilchen gebunden wird (6) und nicht mit dem Abwasser in den Vorfluter gelangt, sind einige der geprüften Flotationsreagenzien so stark toxisch, daß sie auch in Spuren noch vergiftend auf Fische und insbesondere auf Fischnährtiere einwirken können.



Weiterhin muß bei der Beurteilung von Flotationsabwässern auch deren Gehalt an Reglersubstanzen berücksichtigt werden. Spezielle Untersuchungen zu dieser Frage konnten in der vorliegenden Arbeit nicht durchgeführt werden.

Zusammen mit anderen Flotationsreagenzien werden gelegentlich Petroleum oder Dieselöl als Sammler bei der Schwimmaufbereitung verwendet. Von diesen beiden Verbindungen ist jedoch bekannt, daß sie die gesamten biologischen Verhältnisse eines Gewässers schädigen können. Der Schwellenwert für Dieselöl beträgt nach BANDT (7) für Plötzen 25–30 mg l. Die niedere Flora und Fauna wird jedoch schon bei wesentlich geringeren Konzentrationen in Mitleidenschaft gezogen. Den Fischen wird hierdurch die Nahrungsgrundlage entzogen, und sie werden somit zum Abwandern gezwungen.

Zusammenfassend kann gesagt werden, daß die durchgeführten Versuche einen Überblick über die direkte Giftwirkung der verschiedensten Flotationsreagenzien geben. Eine exakte Aussage inwieweit Flotationsabwässer auf fischereilich genutzte Vorfluter schädigend wirken, kann nur nach Bestimmung ihres genauen Gehaltes an Flotationschemikalien gemacht werden. Einige der untersuchten Verbindungen sind jedoch gegenüber Fischen und Fischnährtieren äußerst giftig. Es muß deshalb befürchtet werden, daß selbst bei teilweiser Adsorption an die Feststoffteilchen durch den im Abwasser verbleibenden Rest an Flotationsmitteln die Gefahr einer Schädigung fischereilich genutzter Vorfluter besteht.

## Zusammenfassung

Die mechanische Stofftrennung nach der Flotationsmethode gewinnt in jüngster Zeit für die chemische Industrie mehr und mehr an Bedeutung. In den abgestoßenen Flotationsabwässern sind neben den fischereischädigenden feinen Trübstoffen auch gewisse Mengen der zugesetzten Flotationschemikalien enthalten. Es wurde deshalb die toxikologische Wirkung einer Reihe von Flotationsreagenzien auf Fische und Fischnährtiere untersucht. So wurden Versuche mit verschiedenen Xanthogenaten, Alkylaminen, Terpenkohlenwasserstoffen, Alkylpyridiniumbromiden und Alkylsulfonaten durchgeführt. Weiterhin wurden Ölsäure, p-Tolylarsonsäure, Aerofloat 25, White spirit sowie Tallöl ausgetestet. Als Versuchstiere dienten Plötzen *(Rutilus rutilus)*, Barsche *(Perca fluviatilis)*, Bachflohkrebse *(Gammarus spec.)* und Wasserflöhe *(Daphnia spec.)*. Die Giftwirkung der untersuchten Flotationschemikalien war teilweise recht beträchtlich, so daß Flotationsabwässer, außer durch feine Trübstoffe, unter Umständen auch durch ihre Giftwirkung zu einer Schädigung fischereilich genutzter Vorfluter führen können.

## Summary

Recently mechanical partition by flotation gains more and more importance in chemical industry. The running off flotation effluent contains besides particulate turbids detrimental to fishery a certain quantity of added flotation chemicals. Therefore the toxic efficiency of several flotation reagents to fish and fish-food organism has been examined. Experiments with various xanthogenates, alkylamines, terpene hydrocarbons, alkylpyridinium bromides and alkyl sulfonates were conducted. Furtheremore oleic acid, p-tolylarson acid, aerofloat 25, white spirit and tall oil were put to test. The roach (Rutilus rutilus), perch (Perca fluviatilis), water-flea

(Daphnia spec.) and Gammarus spec. have been used as experimental animals. Partially the toxicity of the examined flotation chemicals was pretty high so that flotation effluents besides particulate turbids also may possibly lead by means of their toxicity to a damage of fishery receiving waters.

Резюме

Механическое отделение веществ по флотационному методу в последнее время в химической промышленности преобретает все больше значения. В отработанных флотационных водах наряду с вредными для рыболовства мутящими веществами содержится и некоторое количество добавляющихся для флотации химических веществ. Поэтому исследовалось токсическое действие ряда флотационных реактивов на рыбу и живой рыбный корм. Так проводились опыты различными ксантогенатами, алкиламинами, углеводородами терпенового ряда, алкилпиридинбромидами и алкилсульфонатами. Кроме того испытывались олеиновая кислота, p-толиларсоновая кислота, аэрофлоат 25, White spirit и таллово масло. Подопытными животными служили плотва (Rutilus rutilus), окунь (Perca fluviatilis), Gammarus spec. и водная блоха (Daphnia spec.). Токсическое действие исследованных флотационных химикатов было отчасти довольно значительное, так что отработанные флотационные воды могут приносить вред рыболовству не только содержащимися в них мутящими веществами, но и своей токсичностью.

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# Industrial Chemical Uses of Polyunsaturated Fatty Acids[1]

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## ABSTRACT

Production of vegetable, animal and marine oils containing more than about 40% unsaturated fatty acids totaled 15,000 million pounds in 1968, almost on the scale of petrochemical production. The greater share (64%) of this nonfossil oil production was directed toward food uses, the remainder toward industrial and animal feed uses. The variety of chemical reactions carried out on these unsaturated fatty acid products include hydrogenation, interesterification, dimerization, sulfation, formation of nitrogen compounds, epoxidation, alkaline cleavage and oxidative ozonolysis. Some of these reactions have been developed at Utilization Research and Development Divisions of the Agricultural Research Service, U.S. Department of Agriculture. Research is continuing in developing new reactions for potential industrial application. An example is reductive ozonolysis of unsaturated fatty esters to produce monofunctional aldehydes and bifunctional aldehyde esters.


## INTRODUCTION

Most scientists readily acknowledge the mammoth proportions of the petroleum industry and of its offshoot, the petrochemical industry. Yet probably few are aware of the comparable magnitude of the industry concerned with oils from nonfossil sources. The 1967 production of petroleum crude products for chemical conversion in the United States was 54,000 million pounds, 70% of it consisting of aliphatic hydrocarbons, at an average value of 3 cents/lb. Although production of nonfossil oils is less, it still amounted to 15,000 million pounds in 1968 at values ranging from 3 cents to 39 cents/lb (Table I). Such nonfossil oils include vegetable, animal and marine oils; they are liquid at ordinary temperatures because of their high proportion of unsaturation. Soybean oil and tallow predominate in the United States, but tall oil and cottonseed oil follow closely.

Most of these oils contain various proportions of oleic, linoleic and linolenic acids, but some contain unusual acids such as ricinoleic, erucic and eleostearic (Table II). Tall oil deserves special mention because of its rapid growth in the past 15-20 years. Its name is derived from the Swedish "tallolja" (pine oil), and it is a byproduct of the sulfate-pulp industry. Distilled tall oil contains 14-37% rosin and 60-85% unsaturated fatty acids, which comprise roughly 50% oleic, 40% linoleic and 6% conjugated acid. These acids are present as the free acid and not as the glyceride.

An overall picture will be drawn for (a) the consumption and use patterns for these oils and for their unsaturated fatty acids; (b) their industrial reactions; (c) the utilization research areas under investigation by the Agricultural Research Service of the U.S. Department of Agriculture; and (d) reductive ozonolysis, an aspect of the oil research at the Northern Regional Research Laboratory. Monounsaturated as well as polyunsaturated fatty acids will be discussed because for most uses it is impractical to separate these acids.

## OIL CONSUMPTION AND USES

By far the greatest market for vegetable oils lies in

[1]Presented at the ISF-AOCS World Congress, Chicago, September 1970.

[2]No. Market. Nutr. Res. Div., ARS, USDA.

TABLE I

Production and Price for Unsaturated Fatty Acid Sources

| Oil or fat | 1968 Production, million lb USA (1) | 1968 Production, million lb World (2) | 1970 Price for crude product, USA cents/lb (3) |
|---|---|---|---|
| Soybean | 6150 | 11,080 | 12 |
| Tallow and grease, edible | 538 | | --- |
| Tallow and grease, inedible | 4745 | 9310 | 7 |
| Cottonseed | --- | 7950 | --- |
| Peanut | 209 | 7010 | 15 |
| Cottonseed | 1115 | 4830 | 13 |
| Rapeseed | --- | 3690 | 17 |
| Palm | --- | 2954 | 38 |
| Palm | 97[a] | 2880 | 13 |
| Fish | 171 | 2440 | 12 |
| Linseed | 307 | 1720 | 11 |
| Sesame seed | --- | 1320 | 39 |
| Castor | 133[a] | 820 | 15 |
| Corn | 453 | 530 | 15 |
| Safflower | 86[a] | 470 | 17 |
| Tung | 32[a] | 268 | 24 |
| Whale | 40[a] | 200 | 15 |
| Oiticica | --- | 78 | 17 |
| Tall oil | 1239 | --- | 3 |
| Vegetable oil foots | 265 | --- | 6 |

[a]Consumption in selected products. Production figures are withheld to avoid disclosing figures for individual companies.

TABLE II

Oil Composition[a]

| Oil or fatty acids | Composition, wt % Oleic | Linoleic | Linolenic | Other |
|---|---|---|---|---|
| **Vegetable oils** | | | | |
| Castor | 3 | 4 | --- | 89[b] |
| Corn | 27 | 59 | 1 | --- |
| Cottonseed | 17 | 53 | --- | --- |
| Crambe | 17 | 9 | 6 | 60[c] |
| Linseed | 19 | 15 | 57 | --- |
| Oiticica | 6 | --- | --- | 78[d] |
| Olive | 52 | 15 | 1 | --- |
| Palm | 38 | 10 | --- | --- |
| Peanut | 51 | 31 | --- | --- |
| Perilla | 13 | 14 | 64 | --- |
| Rapeseed | 17 | 13 | 5 | 56[c] |
| Safflower | 13 | 78 | --- | --- |
| Soybean | 22 | 55 | 8 | --- |
| Sunflower | 14 | 75 | --- | --- |
| Tall oil | 48 | 37 | --- | --- |
| Tung | 8 | 4 | 3 | 80[e] |
| **Animal fats** | | | | |
| Butter fat | 32 | 2 | 1 | 8 |
| Lard | 43 | 9 | --- | 5 |
| Tallow | 44 | 2 | --- | --- |
| **Marine fats and oils** | | | | |
| Herring | 24 | 1 | --- | 44[f] |
| Menhaden | 17 | 1 | --- | 32[f] |
| Sperm whale | 24 | 5 | 2 | 12[f] |

[a]Only those sources having more than 40% total unsaturates are listed.

[b]Ricinoleic acid.

[c]Erucic acid.

[d]Licanic (4-keto-9,11,13-octadecatrienoic) acid.

[e]Eleostearic acid.

[f]Various $C_{18}$ to $C_{24}$ polyunsaturated fatty acids.

TABLE III

1968 Consumption of Oils and Fats in Edible Products, USA, (1) millions of lb

| Oil or fat | Baking or frying fat | Salad or cooking oil | Margarine | Other | Total |
|---|---|---|---|---|---|
| Soybean | 1842 | 2036 | 1240 | 43 | 5161 |
| Cottonseed | 248 | 541 | 70 | 44 | 903 |
| Lard | 601 | 0 | 166[a] | [b] | 767 |
| Tallow, edible | 487 | 0 | 166[a] | [b] | 653 |
| Corn | 10 | 242 | 179 | 8 | 439 |
| Peanut | 21 | 157 | [b] | [b] | 201 |
| Palm | [b] | [b] | [b] | [b] | 97[c] |
| Safflower | 4 | [b] | 45 | [b] | 69 |
| Others | [b] | [b] | [b] | [b] | 17 |
| | | | | | 8307 |

[a]Total for lard and edible tallow.
[b]Consumption figures are withheld to avoid disclosing figures for individual companies.
[c]Total for both edible and inedible products.

various edible products, including baking or frying fats, salad or cooking oils and margarine (Table III). These outlets consume 5161 millions pounds of soybean oil out of a total of 8307 million pounds of all vegetable oils, or 62%.

Consumption of oils in inedible uses amounted to 4700 million pounds in 1968 (Table IV), or 36% of total oil consumption. Although soybean oil is used, tallow and tall oil predominate in the inedible field, especially tallow for feed and both for fatty acids. Tallow is also in demand on a large scale for soaps and for fatty acids. Alkyds and resins and plastics are the major nonfood markets (250 million pounds in 1968) for soybean oil. More and more soybean oil is going into coatings while the amount of linseed oil has declined drastically.

According to the Fatty Acid Producers' Council, the production of unsaturated fatty acids, mainly from tall oil, totaled 570 million pounds in 1969 (Table V). There are five major outlets for tall oil fatty acids, distributed as indicated in Table VI.

The U.S. Tariff Commission has given data for some specific compounds of unsaturated fatty acids (Table VII) (6).

TABLE IV

1968 Consumption in Inedible Products, USA, (1) millions of lb

| Oil | Consumption in major applications | Total consumption |
|---|---|---|
| **Edible oils** | | |
| Soybean[a] | Paints, 79; resins, 102 | 250 |
| Cottonseed | --- | 7 |
| Lard | Lubricants, 12 | 25 |
| Tallow | --- | 8 |
| Peanut | --- | 5 |
| Palm | --- | --- |
| Safflower | Paints, 13; resins, 4 | 19 |
| Others | Resins, 19; lubricants, 5 | 39 |
| | | 353 |
| **Inedible oils** | | |
| Tallow | Feed, 1011; soap, 639; fatty acids, 573; lubricants, 92 | 2478 |
| Tall oil | Fatty acids, 1144; paints, 18; soap, 9; lubricants, 9 | 1288 |
| Linseed | Paints, 122; resins, 19; lubricants, 7 | 194 |
| Castor | Paints, 13; lubricants, 8 | 133 |
| Vegetable oil foots | Fatty acids, 82 | 123 |
| Sperm | Lubricants, 19 | 40 |
| Tung | Paints, 22; resins, 6 | 32 |
| Fish | Paints, 6 | 30 |
| Others | --- | 39 |
| | | 4357 |
| | Overall total | 4710 |

[a]According to USDA (4), nonfood uses for soybean oil totalled 492 million pounds for the year beginning October, 1968. Foots and loss amounted to 243 million pounds; foots are largely consumed in animal feeds and industrial soaps.

## INDUSTRIAL REACTIONS

Many industrial uses cited for vegetable oils depend upon a chemical reaction being carried out on the oil or fatty acid. Some of the more noteworthy reactions are outlined here. For a more thorough treatment consult the literature listed in References 53-59.

### Hydrogenation

Partial hydrogenation is an essential reaction in the production of food items (margarine, salad and cooking oils and shortening), as well as of industrial chemicals. A general reaction can be written in the following way for oleic, linoleic and linolenic acids if it is recognized that extensive geometric and positional isomerizations have occurred in

TABLE V

1969 Production of Unsaturated Fatty Acids[a]

| Fatty acid | Iodine value | 1969 Production, million lb |
|---|---|---|
| Oleic (red oil) | [b] | 130.9 |
| Animal[c] | 36-80 | 32.5 |
| Vegetable or marine | <115 | 8.9 |
| Unsaturated | 116-130 | 17.7 |
| Unsaturated | >130 | 9.3 |
| Tall oil[d] | [b] | 174.2 |
| Tall oil[e] | [b] | 196.5 |
| | | 570.0 |

[a]Fatty Acid Producers' Council.
[b]Iodine value not defined for these acids.
[c]Animal fatty acids other than oleic.
[d]Tall oil fatty acids containing more than 2% rosin acids.
[e]Tall oil fatty acids containing less than 2% rosin acids.

TABLE VI

Tall Oil Fatty Acid Uses, 1967 (5)

| Use | Consumption, million lb |
|---|---|
| Coatings | 79 |
| Dimer acid | 40 |
| Soaps | 33 |
| Flotation agents | 29 |
| Epoxy tallates | 20 |
| Others | 67 |
| | 268 |

TABLE VII

Production, Unit Value and Industrial Use for Selected Unsaturated Fatty Acids (6)

| Unsaturated fatty acid derivative | 1967 Production, million lb | 1967 Unit value, dollars/lb | Industrial use[a] |
|---|---|---|---|
| **Tallow** | | | |
| Salt, potassium | 45.2 | --- | SAA |
| Salt, sodium | 533.1 | 0.13 | SAA |
| Glyceride, sulfated, sodium salt | 10.0 | 0.13 | SAA |
| Amine | | | |
| Tallow alkyl amine | 5.2 | 0.24 | SAA |
| Hydrogenated tallow alkyl amine | 2.6 | 0.28 | SAA |
| Ethoxylated tallow alkyl amine | 1.3 | 0.55 | SAA |
| *N*-(Tallow alkyl) trimethylene diamine | 3.6 | 0.30 | SAA |
| Bis(hydrogenated tallow alkyl) dimethyl ammonium chloride | 20.7 | 0.24 | SAA |
| *N*-Methyl *bis*(hydrogenated tallow alkyl) amine | 2.8[b] | 0.27 | SAA |
| | 624.5 | | |
| **Oleic acid** | | | |
| Salts | | | |
| Sodium | 1.8 | 0.20 | SAA |
| Potassium | 1.1 | 0.23 | SAA |
| Acid, sulfated, disodium salt | 9.7 | --- | SAA |
| Esters | | | |
| Alkyl | 11.0 | 0.21-0.28 | PLA |
| Alkyl, sulfated, sodium salt | 7.9 | 0.28 | SAA |
| Anhydrosorbitol esters (including ethoxylated esters) | 11.7 | 0.38-0.42 | SAA |
| Diethylene and polyethylene glycol esters | 6.9 | 0.29-0.35 | SAA |
| Glycerol monooleate | 2.5 | 0.36 | SAA |
| Amides | | | |
| Ethylene diamine, monoethoxylated | 4.6[c] | --- | SAA |
| Diethanolamides | 2.0 | 0.30-0.65 | SAA |
| Amines | | | |
| Oleylamine | 1.8 | 0.43[c] | SAA |
| *N*-Oleyl trimethylene diamine | 1.6 | 0.37 | SAA |
| Alcohol | | | |
| Oleyl alcohol, ethoxylated | 3.6 | 0.52 | SAA |
| **Tall oil** | | | |
| Salts | | | |
| Sodium | 10.0 | --- | SAA |
| Potassium | 11.7 | --- | SAA |
| Other | 7.8 | 0.23-0.48 | DRI |
| Acids, sulfated sodium salt | 0.8 | 0.24 | SAA |
| Esters | | | |
| Polyethylene glycol esters | 8.5 | 0.17 | SAA |
| Anhydrosorbitol monoester | 0.6 | --- | SAA |
| Amides | | | |
| Diethanolamides | 0.4 | --- | SAA |
| Epoxy | | | |
| 2-Ethylhexyl epoxytallate | 9.7[b] | 0.24 | PLAST |
| Octyl epoxytallate | 15.1 | 0.26 | PLAST |
| **Miscellaneous** | | | |
| Soybean oil, sulfated, sodium salt | 0.3 | 0.36 | SAA |
| Soybean alkyl amine, ethoxylated | 0.9 | 0.37 | SAA |
| Soybean oil, epoxidized | 62.1 | 0.26 | PLAST |
| Castor oil, ethoxylated | 4.3 | 0.33 | SAA |
| Castor oil, sulfated, sodium salt | 7.0 | 0.33 | SAA |
| Sperm oil, sulfated, sodium salt | 6.5 | 0.17 | SAA |
| Sperm oil, sulfurized | 23.0[c] | --- | LUB |
| Azelaic acid esters | 17.5 | 0.29 | PLA |
| Sebacic acid esters | 11.5 | 0.50-0.59 | PLA |
| Erucamide | 1.1 | 1.25 | |

[a]SAA, surface active agents; PLA, plasticizers; DRI, driers for surface coatings; PLAST, plasticizer-stabilizer; LUB, lubricating oil additives.
[b]Sales.
[c]Year of 1966.

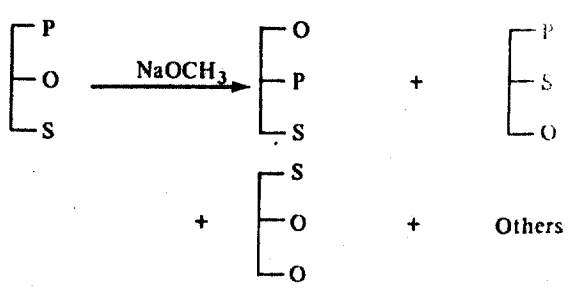
the tially hydrogenated product (G = glyceride):

$$G\text{-}OC(CH_2)_7(CH{=}CHCH_2)_m(CH_2)_nCH_3 \xrightarrow[\text{catalyst}]{H_2} GOOC(CH_2)_x(CH{=}CHCH_2)_y(CH_2)_zCH_3$$

where m=1, 2 or 3; n=0, 3 or 6; 3m+n=9;
y=0, 1 or 2; x+3y+z=16

## Interesterification

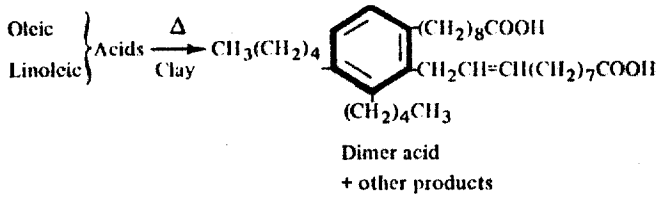
This reaction gives a modified product having a suitable ... range for shortening.




P = palmitic, S = stearic, O= oleic

### Dimerization

Dimer and trimer acids have found outlets in many different commercial applications, most important of which are the dimer acid polyamides used in epoxy adhesives and resins, heat sealing resins and printing inks (7). These acids are also used in making polyesters for polyurethane foams and in making lubricants, corrosion inhibitors and alkyds. They are generally produced by heating oleic acid or tall oil fatty acids over a clay catalyst (8):




Dimer acid
+ other products

### Sulfation

Reaction of sulfuric acid with the double bond is an important one in producing textile lubricants, wetting agents and a fat-liquoring agent for leather. At mild temperatures, sulfation predominates; at higher temperature, sulfonation occurs.

$$RCH{=}CHR' + H_2SO_4 \longrightarrow$$

$$RCH(OSO_3H)-CH(H)R' + \text{other products}$$

### Nitrogen Compounds

Conversion of unsaturated fatty acids to amides, nitriles and saturated amines may be illustrated as follows:

1. $RCOOH + NH_3 \xrightarrow{\Delta} RCONH_2 + H_2O$
2. $RCONH_2 \xrightarrow[\text{Catalyst}]{\Delta} RC{\equiv}N + H_2O$
3. $RC{\equiv}N \xrightarrow{H_2,Ni} RCH_2NH_2$

Amides serve as antiblock agents, as solvents and in waterproofing; nitriles, in low temperature plasticizers and yarn lubricants; and amines, as intermediates for surface active agents and oil flotation.

### Epoxidation

Epoxidized oils useful as a combination plasticizer and stabilizer for poly(vinyl chloride) and as an active reactant for making resins are prepared according to the scheme:

$$GOOC(CH_2)_7(CH{=}CHCH_2)_x(CH_2)_yCH_3 \xrightarrow[H^+]{H_2O_2}$$

$$GOOC(CH_2)_7(CH\text{-}CHCH_2)_x(CH_2)_yCH_3$$

$x = 2$ or 3, $3x + y = 9$

### Alkaline Cleavage

Heating ricinoleic acid or castor oil in the presence of caustic produces sebacic acid, valuable as an intermediate for plasticizers, for polyamides such as nylon-6/10 and for lubricants.

$$CH_3(CH_2)_5CH(OH)CH_2CH{=}CH(CH_2)_7COOH \xrightarrow[250\,(]{NaOH,\ H_2O}$$

$$CH_3(CH_2)_5\underset{OH}{CH}CH_3 + HOOC(CH_2)_8COOH + H_2$$

### Oxidative Ozonolysis

Azelaic acid is produced by oxidative ozonolysis o[f] oleic acid and is used for making poly(vinyl chloride) plast[ics] and lubricants. Pelargonic acid, the other cleavage pr[oduct] is valuable for making jet engine lubricants.

$$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH \xrightarrow[RCOOH]{O_3} [\text{Ozonolysis products}] \longrightarrow$$

$$CH_3(CH_2)_7COOH + HOOC(CH_2)_7COOH$$

## UTILIZATION RESEARCH

Research on unsaturated vegetable and animal fats and oils is carried out by the Agricultural Research Service of the U.S. Department of Agriculture at five different locations (Table VIII). Research on fish oils is conducted by the U.S. Bureau of Commercial Fisheries. A few examples of utilization research accomplishments adopted by industry at sometime or another will be cited here, but no attempt will be made to document them completely. Lists of publications and patents are available from each of the five Divisions. A categorized list of NU publications is available from the Oilseed Crops Laboratory of the Northern Division. Selected investigations will illustrate current activities.

The Eastern Laboratory has developed epoxidized oils valuable as plasticizer-stabilizers for poly(vinyl chloride), biodegradable detergents from tallow alcohol sulfates and α-sulfoacids, vinyl ester polymers and copolymers and potential lubricants and surface active agents from phenyl-stearic acid. Investigations on epoxidation, vinyl monomers, peroxy acids, hydroxy acids and γ-stearolactone, carboxy-stearic acid, alkyl 9(10)-phosphonostearates and sulfur compounds were summarized in 1963 (9). Later investigations include reactions of iospropenyl esters (10), surface active properties of sulfated alkanolamides (11), flame resistant polyurethane foams from hypohalogenated glycerides (12) and potential lubricant additives from *N*-sulfonylaziridine derivatives (13).

The Southern Laboratory has developed water resistant intumescent fire retardant paints from tung oil (14,15) and acetoglycerides for food coatings (16). The acetoglycerides have potential use as plasticizers for synthetic resins including polyvinyl resins (17); and epoxidized acetoglycerides, as stabilizer-plasticizers for vinyl chloride-containing resins (18). Plasticizers based on *N,N*-dimethyl amides (19) and other *N,N*-disubstituted amides (20) have been made, and sucrose ester emulsifiers have been prepared by interesterification in the absence of a solvent (21). This process should make possible the preparation of linoleates of high functionality useful as drying oils.

The Western Laboratory has developed castor oil-based rigid urethane foams (22,23) including some with flame resistant properties (24). Other studies are 10-hydroxy-decanoic acid by alkaline cleavage of ricinoleates (25), dic[arboxylic] ricinoleates from dihaloalkanes for polyurethanes (26), catalytic dehydrogenation of methyl ricinoleate (27) and of methyl 12-hydroxystearate (28,29), synthesis of some acrylate esters of ricinoleates (30), chemical modification of high oleic safflower oil (31), rates of reaction of hexahalocyclopentadienes with long chain olefins (32) and

thermal isomerization equilibrium between conjugated and unconjugated unsaturated keto esters (33).

The Northern Laboratory has developed dimer acid and polyamides and polyesters therefrom, inactivation of metal contaminants in edible oils, selective hydrogenation of edible oils, linseed emulsions for paint and concrete treatment and crambe oil and erucic acid for various industrial purposes. Current reports include hydroformylation of unsaturated fatty esters (34), reactions of azelaldehydic esters obtained by reductive ozonolysis (35), diisocyanate-modified polyesteramides for protective coatings (36), potential lubricant additives of plasticizers from 1,2-cycloaddition of haloalkenes to conjugated fatty esters (37), homogeneous hydrogenation of polyunsaturates to *cis*-monounsaturates with chromium carbonyl (38) and homogeneous catalytic conjugation of polyunsaturated also by chromium carbonyls (39).

## REDUCTIVE OZONOLYSIS

Although oxidative ozonolysis is a well-known industrial reaction, reductive ozonolysis has not yet reached industrial scale. Such nonacceptance may be ascribed to a lack of understanding the reaction, high reactivity of the aldehydic products, poor yields, but most of all to a lack in demonstrated utility for the products. Such products include pelargonaldehyde and methyl azelaaldehydate when reductive ozonolysis is applied to methyl oleate. For several years, this reaction and potential areas for industrial application have been investigated at the Northern Laboratory.

All ozonolyses are performed in two steps, the first step being common to both oxidative and reductive ozonolyses (40). To produce acids, the ozonolysis products are oxidized; for aldehydes, they are reduced. Both solvent and reducing agent profoundly affect the results of ozonolysis. Solvents may be protolytic and react with the intermediate zwitterion or nonprotolytic and nonreactive. The reducing agent may be hydrogen in the present of a catalyst or it may be some other chemical reagent.

Methyl azelaaldehydate may be obtained in about 90% yield when methyl oleate is ozonized in methanol and the ozonolysis products are reduced with zinc and acetic acid (41). A less costly reducing agent is hydrogen. However, when the same ozonolysis products are catalytically hydrogenated over palladium on charcoal, the yield of methyl azelaaldehydate is only about 70% and dimethyl azelate is formed in about 25% yield (42). Dimethyl azelate forms because the methoxy hydroperoxide ozonolysis product decomposes on the catalyst surface:

$$\mathrm{RC(H)(OOH)(OCH_3)} \longrightarrow \mathrm{RC(=O)(OCH_3)} + \mathrm{H_2O}$$

It noteworthy that chain degradation, which has been observed during either oxidation or reduction of other ty of ozonolysis products, does not occur.

ere are at least three ways to minimize hydroperoxide decomposition, probably all of which involve some modification of the catalyst's surface and activity. The first employs methanol containing 10% pyridine as an ozonolysis and hydrogenation solvent. Pyridine reduces the dimethyl azelate formation to 5-10%, prevents acetal formation and inhibits hydrogenation of residual double bonds (43).

Decomposition of methoxy hydroperoxide can also be minimized by a palladium on calcium carbonate catalyst that has been poisoned with lead acetate (Lindlar catalyst) (44). Here again, basic conditions, this time supplied by the support, inhibit acetal formation. The lead acetate inhibits double bond hydrogenation.

TABLE VIII

Location of Government Utilization Research on Unsaturated Fat and Oil Commodities

| Commidity | USA production, 1968, million lb | Location[a] |
|---|---|---|
| Soybeal oil | 6150 | NU |
| Tallow, grease and lard | 5283 | EU |
| Cottonseed oil | 1115 | SU |
| Linseed oil | 307 | NU |
| Fish and whale oil | 211 | BCF |
| Peanut oil | 209 | SU |
| Castor oil | 133[b] | WU |
| Safflower oil | 86[b] | WU |
| Sunflower oil | -- | RRC |
| New crops[c] | -- | NU |

[a]Utilization research is carried out at five Regional Research Laboratories of the Agricultural Research Service, U.S. Department of Agriculture: Northern Utilization Research and Development Division (NU), Peoria, Illinois 61604; Eastern Utilization Research and Development Division (EU), Philadelphia, Pennsylvania 19118; Southern Utilization Research and Development Division (SU), New Orleans, Louisiana 70119; Western Utilization Research and Development Division (WU), Albany, California 94710; and the Richard B. Russell Agricultural Research Center (RRC), (formerly the Southeastern Agricultural Research Laboratory SEU), Athens, Georgia 30601. This new facility is now being staffed and research programs are being implemented. Research on fish oils is carried out at the U.S. Bureau of Commercial Fisheries (BCF), Seattle, Washington.

[b]Consumption in selected products.

[c]New crops research includes the high erucic crambe oil from *Crambe abyssinica*.

The third method employs an ozonolysis solvent consisting of an equimolar mixture of an alcohol and a carboxylic acid (45). The carboxylic acid is preferably acetic acid, but the alcohol can be methyl, ethyl, *n*-butyl alcohol or even 2-methoxyethanol. Hydrogenation proceeds quickly with palladium on charcoal at atmospheric pressure to produce aldehydes in excellent yields (>90%). With this solvent combination, acetal formation can be a problem unless the temperature during both steps is maintained below 15-20 C. Deviation from equimolarity in either direction increases the amount of hydroperoxide decomposition to ester.

The three preceding ozonolyses were all carried out in organic solvents. A more economic ozonolysis medium is water. Surprisingly, water acts as a reactive solvent and is an effective ozonolysis medium for unsaturated fatty esters when the water is present as a water in oil emulsion (46,47). Depending upon hydrogenation conditions and catalysts, aldehyde yield may be as high as 89%. Decomposition of the peroxidic ozonolysis products, probably hydroxy hydroperoxides,

$$\mathrm{RC(H)(OOH)(OH)}$$

also occurs during hydrognation, but the product is a carboxylic acid and not an ester. The byproduct is removed by an alkaline wash.

The aldehydic products have been investigated for a number of purposes, some of which would seen to have considerable merit, e.g., low temperature plasticizers for poly(vinyl chloride) (48), as intermediates for nylon-9 synthesis (49) and as starting materials for several types of coatings (50-52).

## REFERENCES

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# Metabolism of β-Sitosterol in Man

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ABSTRACT The metabolism of β-sitosterol was compared to that of cholesterol in 12 patients. Sterol balance methods were supplemented by radiosterol studies, with the following results. (*a*) Plasma concentrations of β-sitosterol ranged from 0.30 to 1.02 mg/100 ml plasma in patients on intakes of β-sitosterol typical of the American diet. Plasma levels were raised little when intakes were increased greatly, and on fixed intakes they were constant from week to week. On diets devoid of plant sterols, the plasma and feces rapidly became free of β-sitosterol. (*b*) The percentage of esterified β-sitosterol in the plasma was the same as for cholesterol. However, the rate of esterification of β-sitosterol was slower than that for cholesterol. (*c*) Specific activity–time curves after simultaneous pulse labeling with β-sitosterol-$^{3}$H and cholesterol-$^{14}$C conformed to two-pool models. The two exponential half-lives of β-sitosterol were much shorter than for cholesterol, and pool sizes were much smaller. Values of turnover for β-sitosterol obtained by the sterol balance method agreed closely with those derived by use of the two-pool model. There was no endogenous synthesis of β-sitosterol in the patients studied; hence, daily turnover of β-sitosterol equaled its daily absorption. Absorption of β-sitosterol was 5% (or less) of daily intake, while cholesterol absorption ranged from 45 to 54% of intake. (*d*) About 20% of the absorbed β-sitosterol was converted to cholic and chenodeoxycholic acids. The remainder was excreted in bile as free sterol; this excretion was more rapid than that of cholesterol. (*e*) The employment of β-sitosterol as an internal standard to correct for losses of cholesterol in sterol balance studies is further validated by the results presented here.

## INTRODUCTION

The fastidious work of Schönheimer (1, 2) in the 1930's demonstrated that little if any intestinal absorption of plant sterols occurs in mammals. In five animal species Schönheimer found no increase in the total sterol content of the liver after feeding large amounts of plant sterols and concluded that plant sterols were not absorbed to any appreciable extent. These conclusions were sharpened in 1955 by Gould, Jones, Wissler, and Taylor (3, 4), who addressed the question of β-sitosterol absorption in moribund patients and in rats with the greater sensitivity inherent in the use of radioactive sterols; these authors estimated the absorption of β-sitosterol at less than 5% of the dose fed. In rats Borgström (5) in 1968 verified these low levels of absorption of β-sitosterol.

However, later investigators (6–9) reached different conclusions: they measured β-sitosterol absorption in rats by means of various sterol balance procedures and ascribed to absorption any difference between oral intake and fecal excretion. In this manner they ascertained that up to 50% of the intake was absorbable. However, these workers did not consider that losses of plant sterols during intestinal transit can be due to bacterial destruction of the sterol ring, a possibility that to us seems highly likely (10).

Since we have claimed (10) that β-sitosterol is an ideal internal standard to correct for degradative losses of cholesterol in sterol balance studies, we considered it necessary to reevaluate the question of β-sitosterol absorption in man. We measured the absorption of β-sitosterol by the isotopic balance method (11) and by analysis of specific activity–time curves after pulse labeling intravenously (12); we then compared these results with data for cholesterol absorption obtained simultaneously in the same patients. Our findings agree with the results of Gould and of Borgström, namely, that less than 5% of dietary β-sitosterol is absorbed. In the course of these studies we obtained other data concerning the metabolism of β-sitosterol in man, such as the question of its endogenous synthesis (13), its esterification and transport in the plasma, and its transformation into cholic and chenodeoxycholic acids.

## METHODS

### Patients

Studies were carried out on the metabolic ward at The Rockefeller University Hospital; the age, sex, body build, and clinical diagnosis of each patient are given in Table I. Eight patients had familial hypercholesterolemia with normal

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plasma triglyceride concentrations (type II by the lipoprotein phenotyping method of Fredrickson, Levy, and Lees [15]); three patients had elevations in both plasma cholesterol and triglyceride concentrations (one type IV and two type V); and one patient had normal concentrations of lipoproteins.

## Diets

Food intakes consisted exclusively of orally administered liquid formula feedings in which dietary fats contributed 40%, protein 15%, and glucose 45% of total caloric intake, or were fat free (15% protein and 85% glucose); vitamin and mineral supplements were given as described previously (16). In each case the caloric intake was adjusted to maintain total body weight at a constant level throughout each study.

Table II lists the dietary fats used and the sterol contents of the various formulas. When the plant sterols and cholesterol were not inherent in the dietary fats, they were dissolved in them prior to their incorporation in the formulas during large scale homogenization (16). Aliquots of each formula were repeatedly analyzed for sterol content (17) as tests of formula homogeneity.

## Experimental design

(*a*) *Patient 1a.* Patient 1 was studied on different diets in two successive years. In the earlier study (1*a*) a formula diet containing tracer amounts of β-sitosterol-22,23-$^3$H was fed five times daily for 83 days in order to attain the isotopic steady state (18) for β-sitosterol. During this steady state, measurements of the concentration of β-sitosterol in the plasma were found to be the same by two independent methods, gas-liquid chromatographic (GLC), and isotopic (see section Isotopes). After oral administration of labeled β-sitosterol was discontinued, the cumulative clearance of β-sitosterol from the body was determined as a measure of the amount of the sterol retained in the body.

(*b*) *Patients 1b, 2, and 3.* The rates of turnover of β-sitosterol and cholesterol in the plasma were determined from specific activity–time curves in patients 1*b*, 2, and 3 after simultaneous pulse labeling with β-sitosterol-22,23-$^3$H and cholesterol-4-$^{14}$C by the intravenous route; calculations of body pool sizes and intestinal absorption of cholesterol and β-sitosterol also were made. Conversion of β-sitosterol into the primary bile acids was demonstrated on samples of bile obtained by duodenal intubation.

(*c*) *Patients 4–8.* In these five patients the effects of moderate and high intakes of dietary β-sitosterol (designated as studies *a* and *b*, respectively) on plasma concentrations of cholesterol and β-sitosterol were assessed. Measurements of turnover and pool sizes of β-sitosterol were made in patient 4 on the two intake levels.

(*d*) *Patients 9–12.* Stools from these patients were analyzed for the presence of β-sitosterol at a time when this sterol had been absent from the diets for at least 4 wk. Measurements were made by gas-liquid chromatography (GLC), utilizing the maximum sensitivity that we can presently attain (see under Results).

TABLE I
*Clinical Data*

| Patient No. | Initials | Age | Sex | Height | Weight | % of Ideal Weight* | Diagnosis‡ |
|---|---|---|---|---|---|---|---|
| | | *yr* | | *cm* | *kg* | | |
| 1 | H. T. | 57 | M | 162 | 46 | 84 | Hypercholesterolemia (Type II) |
| 2 | M. R. | 60 | M | 167 | 67 | 109 | Hyperglyceridemia (Type V) |
| 3 | C. Z. | 63 | M | 181 | 94 | 122 | Normocholesterolemia, IHD,§ PVD§ |
| 4 | M. S. | 37 | M | 167 | 54 | 93 | Hypercholesterolemia (Type II), IHD, xanthomatosis |
| 5 | J. R. | 36 | F | 164 | 53 | 98 | Hypercholesterolemia (Type II), IHD, PVD, xanthomatosis |
| 6 | R. G. | 58 | F | 147 | 61 | 120 | Hypercholesterolemia (Type II), IHD, xanthomatosis, essential hypertension |
| 7 | J. H. | 39 | M | 172 | 74 | 104 | Hypercholesterolemia (Type II) |
| 8 | N. A. | 30 | M | 170 | 67 | 102 | Hypercholesterolemia (Type II), IHD |
| 9 | A. M. | 60 | F | 164 | 66 | 108 | Hyperglyceridemia (Type IV), IHD, PVD, chronic lymphatic leukemia |
| 10 | E. K. | 45 | F | 159 | 56 | 105 | Hypercholesterolemia (Type II), IHD |
| 11 | R. W. | 41 | M | 176 | 75 | 102 | Hyperglyceridemia (Type V), PVD |
| 12 | R. F. | 55 | F | 154 | 61 | 128 | Hypercholesterolemia (Type II), IHD |

* According to life insurance tables (14).
‡ Phenotyping of hyperlipoproteinemia by paper strip electrophoresis according to Fredrickson, Levy, and Lees (15).
§ IHD, ischemic heart disease; PVD, peripheral vascular disease.

TABLE II

*Intakes of Dietary Fats, Sterols, and Total Calories*

| Patient | Cal/day* | Dietary fat‡ and duration of study (*days*) | Period I | | Period II | |
|---|---|---|---|---|---|---|
| | | | Cholesterol | β-Sitosterol | Cholesterol | β-Sitosterol |
| | | | *mg/day* | *mg/day* | *mg/day* | *mg/day* |
| 1*a*§ | 2000 | Butter oil (I-40; III-36) Corn oil (II-83) | 285 | 0 | 35 | 620 |
| 1*b* | 1800 | Lard (129) | 402 | 242 | — | — |
| 2 | 2380 | Lard (134) | 565 | 342 | — | — |
| 3 | 2400 | Lard (123) | 685 | 415 | — | — |
| 4 | 2280 | Cottonseed oil (170) | 34 | 320 | 34 | 1909 |
| 5 | 1875 | Butter oil (176) | 285 | 125 | 315 | 7166 |
| 6 | 2062 | Cottonseed oil (170) | 33 | 293 | 33 | 6488 |
| 7 | 2688 | Butter oil (184) | 452 | 192 | 420 | 6211 |
| 8 | 2375 | Butter oil (224) | 378 | 166 | 380 | 6484 |
| 9 | 2100 | None (42) | 35 | 0 | — | — |
| 10 | 1900 | Butter oil (40) | 50 | 0 | — | — |
| 11 | 2800 | None (55) | 30 | 0 | — | — |
| 12 | 2000 | Egg yolk fat (83) | 2100 | 0 | — | — |

* To maintain constant body weight.

‡ In fat-containing formulas fat calories = 40% of total caloric intake.

§ Patient 1 was studied on two occasions in successive years. In the earlier study he was fed β-sitosterol-22,23-$^3$H daily during period II; in periods I and III he was fed a butter oil formula free of β-sitosterol.

‖ This butter oil was subjected to molecular distillation for removal of free cholesterol (Distillation Products Industries, Rochester, N. Y.).

## Isotopes

β-sitosterol-22,23-$^3$H was prepared according to the method of Steele and Mosettig (19). In this procedure the starting material, i-stigmasterol methyl ether, was prepared by Dr. M. J. Thompson, USDA, Beltsville, Md.; it was reduced with tritium gas by Dr. G. Gupta of The Rockefeller University, and the reduction product was converted into the acetate of β-sitosterol-22,23-$^3$H by Dr. Thompson. We hydrolyzed the sterol ester and isolated the free labeled sterol by thin-layer chromatography (TLC) on Silica Gel G with heptane:diethyl ether, 2:1. The final product had a specific activity of 400 mCi/mmole. Cholesterol-4-$^{14}$C was obtained from New England Nuclear Corp., Boston, Mass. Both labeled sterols were shown to be better than 99% pure by TLC on Florisil (Floridin Company, Tallahassee, Fla.), in the system diethyl ether:*n*-heptane, 55:45. Measurements of radioactivity were made in a Packard Tri-Carb Scintillation Counter, model 3003, (Packard Instrument Co., Inc., Downers Grove, Ill.) as previously described (17).

Patient 1*a* ingested β-sitosterol-22,23-$^3$H (21.3 μCi, 620 mg) daily for a period of 83 days. At the time of homogenization the tracer sterol dissolved in 10 ml of ethanol was added to 40-kg batches of formula containing nonlabeled β-sitosterol in corn oil. A constant intake of isotopically labeled sterol was assured by feeding the same amount of formula each day (five divided doses).

Patients 1*b*, 2, and 3 were given a mixture of β-sitosterol-22,23-$^3$H (136 μCi) and cholesterol-4-$^{14}$C (90 μCi) as a single intravenous pulse at the beginning of their studies. Patient 4 was given a mixture of β-sitosterol-22,23-$^3$H (50 μCi) and cholesterol-4-$^{14}$C (27 μCi) during the first study, and β-sitosterol-22,23-$^3$H (110 μCi) and cholesterol-4-$^{14}$C (90 μCi) during the second study. In all cases the radiosterols were dissolved in 1 ml of ethanol which was then dispersed in 150 ml of physiologic saline; the dispersion was immediately infused by vein.

## Steroid analyses

*Fecal steroids.*[1] Complete stool collections were made throughout each study and combined into 4-day pools. Fecal neutral and acidic steroids were isolated separately, and their masses and specific activities were measured by methods developed in this laboratory (17, 20). These TLC and GLC procedures permit the essential distinction to be made between plant sterols and cholesterol, and between the two families of bacterial conversion products derived from plant sterols and cholesterol during intestinal transit (5β,3β-OH- and 5β,3-keto-compounds).

*Plasma, erythrocyte, and bile steroids.* For analysis of cholesterol and β-sitosterol in plasma, erythrocytes (RBC), and bile, it proved expedient to modify the fecal neutral steroid procedure in several ways. Because the accuracy of the results in this study depends upon the analysis of small amounts of β-sitosterol in the presence of large amounts of cholesterol, we present this procedure in some detail.

1 ml of plasma, packed RBC, or bile was refluxed for 1 hr with 20 ml of N NaOH in 90% ethanol; after refluxing, water (10 ml) was added to the alkaline saponification mixture, and nonsaponifiable components were extracted three times with 50-ml portions of petroleum ether, 60-80°C (PE). The combined PE extracts were evaporated to dryness in a round-bottom flask, and the residue was redissolved in

[1] The term *steroid* is used in preference to *sterol* because significant amounts of ketonic metabolites of cholesterol and of the plant sterols are usually present in the neutral and acidic fractions of the fecal lipids.

5.00 ml of ethyl acetate containing 350 μg of 5α-cholestane. 4 ml of this solution was utilized for radioactivity counting. The remaining 1 ml was taken for sterol analysis by GLC; solvent was evaporated and the trimethylsilyl (TMS) ethers of the sterols were formed by addition of 50 μl of TMS reaction mixture (Sil Prep, Applied Science Laboratories, State College, Pa.). Generally, about 3 μl of this mixture was analyzed by GLC; this was equivalent to about 1% (1/5 × 3/50) of the neutral sterols in the original 1 ml sample of plasma, packed RBC, or bile.

Much greater sensitivity was required for analyses of materials obtained from patients fed diets free of β-sitosterol. In these cases the neutral sterol fraction was extracted as described above from 1 ml of plasma or fecal homogenate and dissolved in 1.00 ml ethyl acetate containing 70 μg of 5α-cholestane. This in turn was concentrated in the tip of a small conical tube during the evaporation of solvent; TMS ethers were formed by addition of 5 μl of TMS reaction mixture. As much of this mixture as possible was then injected into the GLC column, and the analysis of the β-sitosterol derivative was accomplished at the maximum instrument sensitivity consistent with acceptable base line noise. Under our conditions a peak for TMS-β-sitosterol 2 × base line noise was equivalent to no more than 65 ng per ml of plasma or per g of fecal homogenate.

Quantitative analysis of TMS ethers of cholesterol and β-sitosterol was carried out by GLC on 4-ft columns packed with 1% DC-560 (Applied Science Laboratories) at a column temperature of 240°C with a flame ionization detector, F and M Biomedical Gas Chromatograph, Model 400 (F and M Scientific Corp., Avondale, Pa.); these columns had 2000–3000 theoretical plates for TMS-cholesterol. Individual peak areas were measured by electronic integration, Model CRS-100, (Infotronics, Inc., Houston, Tex.). Because of the large excess of cholesterol relative to β-sitosterol in all samples, the sensitivity of the GLC detector was usually increased 32-fold after emergence of the cholesterol peak. The two sterols were individually quantified by relating their peak areas to that of the internal recovery standard, 5α-cholestane; correction factors are not required (17). In tests with standard mixtures of these two sterols, we found we could reliably measure 65 ng of β-sitosterol per ml of plasma or per g of fecal homogenate, in the presence of large excesses of cholesterol.

In the analysis of bile, neutral sterols were removed by extraction with PE, and the unconjugated bile acids were isolated from the aqueous alcohol phase after alkaline hydrolysis of the conjugated acids according to our method for isolation of fecal acidic steroids (20). The methyl esters of the trihydroxy- and dihydroxycholanic acids were isolated by TLC on Silica Gel H in the system chloroform: acetone: methanol, 70:25:5, and the specific activity of each bile acid class was measured. For quantification of bile acids by GLC, 6-ft columns containing 1% HiEff 8BP (Applied Science Laboratories) were used at 230°C; they offered about 3000 theoretical plates for TMS chenodeoxycholic methyl ester, with a separation factor of 1.10 between this derivative and that of deoxycholic acid.

*Free and esterified sterols in plasma.* The lipids in 2 ml of plasma were extracted into 38 ml of chloroform: methanol, 2:1. During evaporation of solvent small quantities of ethanol were added to facilitate the removal of water; the residue was redissolved in exactly 15.00 ml of ethyl acetate. 10.00 ml was taken for TLC on 0.5 mm Silica Gel H plates; the developing system contained petroleum ether: ethyl ether: acetic acid, 85:15:0.5, in which free sterols have an Rf of 0.30 and esterified sterols 0.90. The free and esterified sterols were collected separately according to the method of Goldrick and Hirsch (21), and measurement of sterol content was made by GLC after saponification and TMS-ether formation, as described earlier in this paper.

## Calculations

*Corrections for neutral steroid losses.* Corrections for losses of neutral steroids during intestinal transit and for variations in fecal flow rates were made with nonlabeled dietary plant sterols as internal standard (10).

*Cholesterol absorption.* The absorption of dietary cholesterol was measured as the difference between dietary intake and unabsorbed dietary neutral steroids in feces. The latter quantity was obtained by the procedure designated as Method I in this laboratory (22): unabsorbed dietary neutral steroids equal total fecal neutral steroids (determined by chromatographic methods) minus fecal neutral steroids of endogenous origin (determined by the isotopic balance technique after intravenous pulse labeling with radioactive cholesterol).

## RESULTS

*β-Sitosterol in feces of patients fed diets free of β-sitosterol.* If β-sitosterol is synthesized by the body, it should be excreted in the feces continuously; the magnitude of this endogenous synthesis will be most clearly defined when the diet is free of plant sterols. With these considerations in mind we attempted to measure β-sitosterol in the feces of patients 9–12 who had been fed formula diets free of this sterol for periods longer than 4 wk. Diets and feces were analyzed, and no β-sitosterol or its bacterial transformation products could be detected.

Since 65 ng of β-sitosterol per ml of plasma or per g of fecal homogenate can be reliably measured by our GLC techniques, we can conclude that excretion of β-sitosterol in patients 9–12 was less than 10 μg/day (the daily weight of feces of these patients ranged from 70 to 150 g). Thus, it appears improbable that these patients synthesized more than 10 μg of β-sitosterol per day.

*Measurement of cholesterol and β-sitosterol concentrations in plasma and RBC.* Table III presents the results obtained for plasma and RBC levels of cholesterol and β-sitosterol in patients 1*b*, 2, and 3 who were fed diets in which the amounts of cholesterol and β-sitosterol approximated those of the average American diet. Measurements of plasma cholesterol and β-sitosterol concentrations were made twice weekly for 9 wk, as well as measurements of levels of RBC sterols. The average concentrations of plasma cholesterol ranged from 226 to 300 mg/100 ml, and of plasma β-sitosterol from 0.30 to 1.02; thus, the ratios of cholesterol to β-sitosterol in plasma ranged from about 300–800 to 1. The relatively small standard deviations of the means for plasma concentrations of cholesterol and β-sitosterol indicate a high degree of constancy of plasma levels of these two sterols throughout each study. RBC concentrations of cholesterol varied from 105 to 131 mg/100 ml of packed

TABLE III

*Concentrations of β-Sitosterol and Cholesterol in Plasma and RBC as Measured by GLC*

| Patient | β-Sitosterol | | | Cholesterol | | | Cholesterol/β-sitosterol | |
|---|---|---|---|---|---|---|---|---|
| | Diet | Plasma | RBC | Diet | Plasma | RBC | Plasma | RBC |
| | *mg/day* | *mg/100 ml** | *mg/100 ml packed cells** | *mg/day* | *mg/100 ml** | *mg/100 ml packed cells** | | |
| 1*b* | 242 | 1.02 ±0.06 (18) | 0.40 ±0.05 (9) | 402 | 287 ±10 (18) | 131 ±9 (9) | 286 | 328 |
| 2 | 342 | 0.51 ±0.02 (18) | 0.17 (2) | 565 | 287 ±9 (18) | 105 (2) | 564 | 617 |
| 3 | 415 | 0.30 ±0.05 (18) | 0.17 (2) | 685 | 226 ±12 (18) | 129 (2) | 786 | 758 |
| 4*a* | 320 | 0.90 ±0.07 (20) | 0.29 (2) | 34 | 300 ±10 (20) | 120 (2) | 350 | 413 |
| 4*b* | 1909 | 1.73 ±0.15 (19) | 0.88 (3) | 34 | 254 ±6 (17) | 129 (3) | 150 | 147 |
| 5*a* | 125 | 0.80 | | 285 | 448 | | 560 | |
| 5*b* | 7166 | 1.46 | | 315 | 354 | | 242 | |
| 6*a* | 293 | 0.73 | | 33 | 267 | | 366 | |
| 6*b* | 6488 | 1.25 | | 33 | 198 | | 158 | |
| 7*a* | 192 | trace | | 452 | 196 | | — | |
| 7*b* | 6211 | 0.46 | | 420 | 156 | | 339 | |
| 8*a* | 166 | 0.42 | | 378 | 132 | | 314 | |
| 8*b* | 6484 | 0.60 | | 380 | 102 | | 170 | |

* Mean ±SD. Figures in parentheses indicate number of determinations.

cells, and β-sitosterol concentrations from 0.17 to 0.40 mg/100 ml of packed cells. The cholesterol to β-sitosterol ratios in RBC were nearly the same as those in plasma, suggesting a free exchange of both sterols between plasma and RBC.

In five patients (patients 4–8) who received β-sitosterol at two levels of intake, concentrations of β-sitosterol in plasma were consistently higher during the period of the higher intake; but, despite eightfold or greater increases in intakes of β-sitosterol, plasma concentrations increased less than twofold. The increase in plasma β-sitosterol during the greater intake was associated with a similar increase in RBC β-sitosterol (patient 4*b*), and again the ratio of cholesterol to β-sitosterol in RBC was the same as that of plasma.

A comparison of the degree of esterification of β-sitosterol with that of cholesterol is presented in Table IV. Three patients (1*b*, 2, and 3) were pulse labeled simultaneously by the intravenous route with β-sitosterol-$^3$H and cholesterol-$^{14}$C, and the distribution of labeled sterols in the free and esterified sterol fractions of plasma was analyzed 30–39 days after administration of isotopes; labeled β-sitosterol was found to be esterified to the same extent as labeled cholesterol (60–75% esterified). In patient 4, measurements of the free and ester ratios of the two radiosterols were begun earlier. Differences in esterification rate were most noticeable during the 1st few days; in this patient β-sitosterol appeared to be esterified at a slower rate than cholesterol.

*The attainment of the isotopic steady state for β-sitosterol.* In another experiment designed to examine whether β-sitosterol was synthesized endogenously, patient 1*a* received a diet free of β-sitosterol (40% butter oil) for 40 days in period I. After 1 wk and throughout the remaining 5 wk on the β-sitosterol-free formula, we found less than 65 ng of β-sitosterol per ml of plasma or per g of feces.

In period II (83 days) the patient was given a formula diet containing 40% of calories as corn oil in which β-sitosterol-22,23-$^3$H had been incorporated during its manufacture; each day the patient ingested 620 mg of β-sitosterol of specific activity 74,245 dpm/mg. On the

2nd day of this regimen radioactivity was detected in the plasma; it rose to reach a constant level of approximately 1000 dpm/ml plasma in about 2 wk (Fig. 1). The plateau in plasma radioactivity persisted for the remainder of period II, suggesting the attainment of the isotopic steady state (18).

During this period when plasma levels of radioactivity were constant, the average specific activity of plasma β-sitosterol (calculated from plasma radioactivity and mass measurement of β-sitosterol by GLC) was 76,149 dpm/mg for the last four determinations (Table V). This agrees within 3% with that of the diet (74,245 dpm/mg). The near identity of specific activities of plasma and dietary β-sitosterol is added evidence that this patient did not synthesize β-sitosterol; if synthesis had occurred, the specific activity of plasma β-sitosterol would have been less than that of the diet, because of dilution of radioactive β-sitosterol in the plasma with that newly synthesized. Furthermore, during the same 16 day period, measurements of the specific activity of the fecal neutral steroids derived from β-sitosterol agreed with those of plasma and dietary β-sitosterol specific activities within ±5% (77,840 dpm/mg ±1743: mean ±SD in five successive 4-day stool collections).

These findings, especially when coupled with the absence of β-sitosterol in plasma and feces during the β-sitosterol–free diet of periods I and III, are taken to indicate that endogenous synthesis of β-sitosterol did not occur.

TABLE IV

*Distribution of β-Sitosterol-$^3$H and Cholesterol-$^{14}$C in Free and Ester Fractions of Plasma*

| Patient | Time after isotope | β-Sitosterol | | Cholesterol | |
|---|---|---|---|---|---|
| | | Free | Ester | Free | Ester |
| | *days* | % | | % | |
| 1b | 35 | 31 | 69 | 28 | 72 |
| | 39 | 33 | 67 | 27 | 73 |
| 2 | 30 | 40 | 60 | 36 | 64 |
| | 35 | 39 | 61 | 33 | 67 |
| 3 | 30 | 28 | 72 | 26 | 74 |
| | 35 | 30 | 70 | 28 | 72 |
| 4a | 4 | 40 | 60 | 32 | 68 |
| | 5 | 35 | 65 | 29 | 71 |
| | 12 | 28 | 72 | 28 | 72 |
| | 15 | 29 | 71 | 29 | 71 |
| 4b | 1 | 70 | 30 | 56 | 44 |
| | 2 | 53 | 47 | 43 | 57 |
| | 5 | 39 | 61 | 34 | 66 |
| | 6 | 31 | 69 | 34 | 66 |
| | 10 | 31 | 69 | 30 | 70 |
| | 14 | 29 | 71 | 30 | 70 |

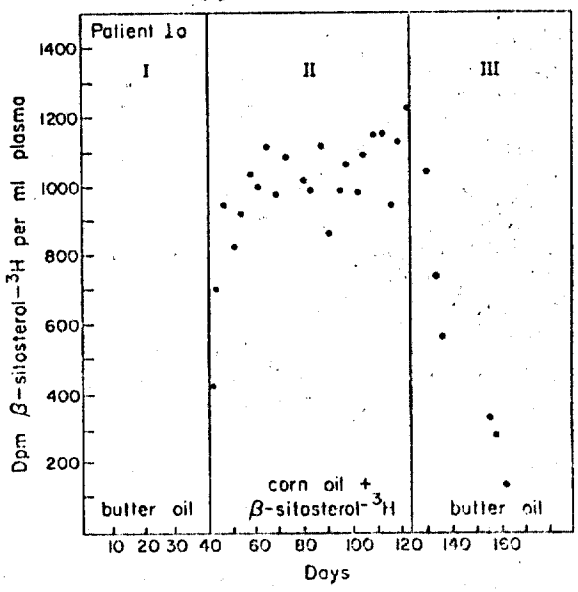


FIGURE 1 Isotopic steady state for β-sitosterol-$^3$H (patient 1a). In period I a butter oil formula free of β-sitosterol was fed; the plant sterol was not detectable in plasma or feces after 3 wk. During period II a corn oil formula that furnished 620 mg/day of β-sitosterol-$^3$H was fed; the isotopic steady state was attained in about 30 days. When the intake of β-sitosterol was discontinued (period III), the radioactivity in the plasma rapidly declined, and in 4 wk unlabeled β-sitosterol was no longer detectable in plasma and feces. In period III, 226 mg of β-sitosterol recovered in the feces was demonstrated to represent the total body pool of β-sitosterol in period II.

In period III β-sitosterol feeding was abruptly discontinued by returning to the butter oil formula of period I. Plasma radioactivity declined sharply from 1000 to less than 150 cpm/ml of plasma after 30 days; by GLC the mass of β-sitosterol in plasma and feces fell to the base line limits noted in period I (Fig. 1). In the first 16 days of period III 369 mg of β-sitosterol was excreted in the feces; thereafter, our most sensitive assays failed to detect this sterol. We estimate that 143 mg of 369 mg represented β-sitosterol which lagged behind in the bowel after its feeding in period II had been discontinued.[3] The difference (369 − 143 = 226 mg) was

[3] Chromic oxide (300 mg/day) had been fed as an inert marker during period II (23). The ingestion of β-sitosterol and chromic oxide was discontinued simultaneously at the end of Period II; thereafter, 69 mg of chromic oxide was excreted in the feces in the first 4 days of period III and none thereafter. Since 69 mg of chromic oxide is equivalent to 23% of 1 day's intake, we have assumed that 23% of the last day's intake of β-sitosterol during period II was excreted in the feces during the 1st days of period III; 23% of 620 mg (1 day's intake) = 143 mg.

TABLE V

*Plasma β-Sitosterol Concentrations Determined by GLC and Isotopic Methods in Patient 1a, Fed β-Sitosterol-22,23-$^3$H Daily for 83 Days (Period II)*

| Time after start of Period II | Plasma β-Sitosterol: Concentration measured by GLC | Radioactivity | Specific activity | Concentration* calculated isotopically | Difference in concentration (GLC vs. isotopic calculation) |
|---|---|---|---|---|---|
| *days* | *mg/100 ml* | *dpm/100 ml* | *dpm/mg* | *mg/100 ml* | *%* |
| 8 | 0.91 | 79,100 | 86,923 | 1.06 | −17 |
| 11 | 1.11 | 83,200 | 74,955 | 1.12 | −1 |
| 15 | 1.37 | 93,500 | 68,248 | 1.26 | +8 |
| 18 | 1.22 | 88,900 | 72,868 | 1.20 | +2 |
| 29 | 1.38 | 104,100 | 75,434 | 1.40 | −1 |
| 32 | 1.26 | 98,000 | 77,778 | 1.32 | −5 |
| 36 | 1.28 | 100,500 | 78,516 | 1.35 | −5 |
| Average of last 4 values | 1.29 ±0.06 | 97,875 ±6,486 | 76,149 ±2,551 | 1.32 ±0.08 | |

* Plasma β-sitosterol concentration calculated by dividing plasma radioactivity (dpm/100 ml) by specific activity of dietary β-sitosterol (74,245 dpm/mg).

taken to represent the total body pool of β-sitosterol fluxing from the body after removal of β-sitosterol from the diet.

The fact that no further β-sitosterol was excreted in the feces or found in the plasma after 30 days on a β-sitosterol–free diet is offered as evidence that the synthesis of β-sitosterol in this man was not repressed by the β-sitosterol absorbed in period II.

TABLE VI

*Measurement of β-Sitosterol Turnover by the Isotopic Balance Method after Pulse Labeling with β-Sitosterol-$^3$H*

| Patient | Neutral steroids* | Acidic steroids* | Turnover (neutral + acidic) |
|---|---|---|---|
| | *mg/day* | *mg/day* | *mg/day* |
| 1*b* | 7.82 (4) | 2.25 | 10.07 |
| 2 | 7.15 (4) | 1.45 | 8.60 |
| 3 | 6.34 (4) | 2.31 | 8.65 |
| 4*a* | 5.01 (6) | 0.17 | 5.18 |
| 4*b* | 13.91 (6) | 0.08 | 13.98 |

* mg/day = dpm/day in each fraction of fecal steroids ÷ dpm/mg of plasma β-sitosterol 2 days earlier. This 2 day interval represents the average transit time for intestinal contents to pass through the intestine (23).

*Analysis of β-sitosterol turnover by the isotopic balance method.* The turnover of β-sitosterol in patients 1*b*, 2, 3, 4*a*, and 4*b* was measured by the isotopic balance method after pulse labeling with β-sitosterol-22,23-$^3$H. This calculation was based on the assumption (proven true in section 3) that the specific activity of the fecal neutral steroids derived from β-sitosterol is the same as the specific activity of plasma β-sitosterol. Thus, the daily excretion (milligrams per day) of neutral and acidic steroids derived from β-sitosterol can be calculated by dividing the total number of disintegrations per minute of tritium excreted per day in each fecal steroid fraction by the specific activity (dpm/mg) of plasma β-sitosterol.

The values thus obtained are shown in Table VI: 5-14 mg/day were excreted as neutral steroids and 0.08-2.31 mg/day as acidic steroids. In the metabolic steady state the sum of the fecal neutral and acidic steroids reflects the daily turnover of β-sitosterol (since all other excretion routes are trivial in man except in biliary obstruction, in which the excretion of bile acids via the kidney may be sizable).

The isotopic balance method also provides a measure of daily bile acid synthesis from β-sitosterol, for in the steady state the daily excretion of fecal acidic steroids is equivalent to their daily synthesis. Patients 1*b*, 2, and 3 formed 2.25, 1.45, and 2.31 mg of bile acids from β-sitosterol each day, respectively. In contrast, this conversion was almost immeasurable in patient 4; as shown, this patient also converted very small amounts of cholesterol into bile acids.

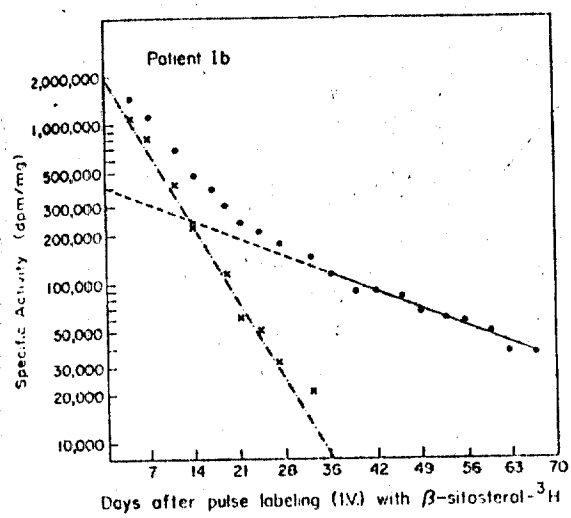


FIGURE 2 Specific activity–time curve for plasma β-sitosterol after pulse labeling intravenously with β-sitosterol-$^3$H. A two pool model is suggested by the curve-peeling method: the difference values (small crosses) obtained by subtracting the points on the extrapolated line from the experimental points fit a straight line.

*Analysis of sterol turnover and pool sizes by the two pool model.* In the four patients pulse labeled by simultaneous intravenous administration of β-sitosterol-22,23-$^3$H and cholesterol-4-$^{14}$C, specific activities of each of the sterols in the plasma were measured twice weekly. Specific activity-time curves for each of the sterols in all four patients showed two components: a rapid nonlinear decline followed by a period of more gradual, but log linear decay (Figs. 2, 3). When these four sets of curves were analyzed mathematically by the equations given by Goodman and Noble (12), each curve conformed to that described for a two pool model. Accordingly, we have calculated values for turnover and pool sizes for each of the sterols (Tables VII, and VIII).

The curves illustrated in Fig. 3 show that in each case the turnover of β-sitosterol was more rapid than that of cholesterol. This comparison is given numerically in Tables VII and VIII: the average value for the $t_{\frac{1}{2}}$ of the first exponential for cholesterol was 1.6 times longer than that of β-sitosterol; the $t_{\frac{1}{2}}$ for the second exponential was 3.5 times longer than that of β-sitosterol. The more rapid disappearance of labeled β-sitosterol previously demonstrated by Gould et al. (3) can be ascribed in part to the smaller body pool of β-sitosterol; however, in the next section we shall present evidence that β-sitosterol actually is excreted preferentially.

In these four patients the plasma β-sitosterol concentration varied from 0.3 to 1.73 mg/100 ml, a fivefold variation. Yet, the narrow range of variation of the two exponentials of radioactive β-sitosterol indicates that the kinetics of β-sitosterol turnover were very much alike from patient to patient. Indeed, in patient 4 who was tested during periods of low and high dietary intakes of β-sitosterol, the two sets of half-lives of β-sitosterol were almost identical, even though the plasma concentration of β-sitosterol had doubled.

*Estimates of β-sitosterol absorption.* When the values for turnover of β-sitosterol derived by the two pool model (Table VII) were compared with the values obtained by the isotopic balance method (Table VI), close agreement was observed. Since all available evidence leads us to conclude that β-sitosterol was not synthesized by our patients, it becomes clear that in the metabolic steady state the daily turnover of β-sitosterol equals the amount absorbed each day from dietary sources (Fig. 4). When these four patients ingested 242 to 415 mg of β-sitosterol per day, the absorption of β-sitosterol varied from 6.5 to 12.5 mg/day according to the two pool model, and 5.2 to 14 mg/day according to the isotopic balance method; the differences are not significantly different (Student's $t$ test). Patient 4 absorbed 5.2–7.0 mg/day out of a daily intake of 320 mg, and and 13.5–14.0 mg when the diet contained 1900 mg/day, a doubling of absolute absorption with a sixfold increase in intake.

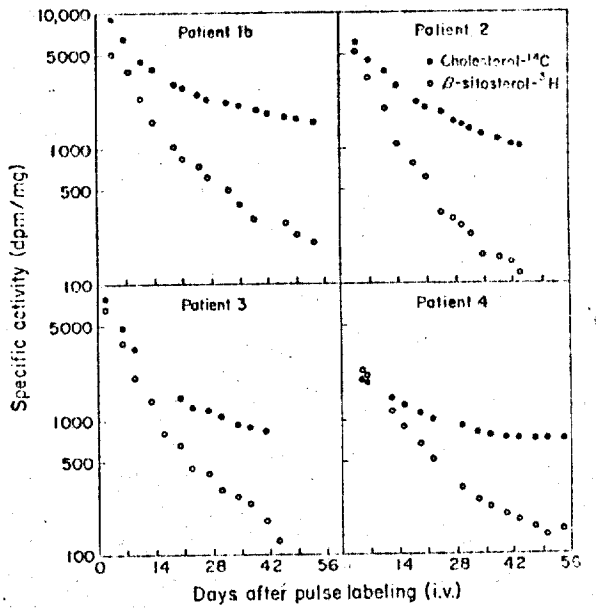


FIGURE 3 Comparative specific activity–time curves for plasma sterols after pulse labeling intravenously with β-sitosterol-$^3$H and cholesterol-$^{14}$C simultaneously in four patients. In each case the decay of β-sitosterol was more rapid than for cholesterol, indicating a more rapid turnover of β-sitosterol.

TABLE IX

*Equilibration of Labeled Sterols Between Plasma, RBC, and Bile after Pulse Labeling Intravenously*

| Patient | Time after Pulse Labeling | β-sito.-$^3$H SA* / chol.-$^{14}$C SA | $^3$H/$^{14}$C | β-sito.-$^3$H SA / chol.-$^{14}$C SA | $^3$H/$^{14}$C | β-sito.-$^3$H SA / chol.-$^{14}$C SA | $^3$H/$^{14}$C |
|---|---|---|---|---|---|---|---|
| | *days* | | *dpm ratio per ml plasma* | | *dpm ratio per ml packed cells* | | *dpm ratio per ml bile* |
| 1b | 27 | 313,732<br>3,023 | 0.40 | — | — | 235,143<br>2,829 | 1.00 |
| | 34 | 255,219<br>2,477 | 0.35 | 281,335<br>2,491 | 0.33 | 266,929<br>2,438 | 0.95 |
| | 41 | 140,707<br>2,097 | 0.27 | 153,457<br>2,056 | 0.32 | 130,576<br>1,877 | 0.82 |
| | 49 | 101,984<br>1,879 | 0.24 | 132,990<br>1,865 | 0.24 | 92,737<br>1,915 | 0.64 |
| | 56 | 90,156<br>1,602 | 0.20 | 116,909<br>1,540 | 0.20 | 86,617<br>1,525 | 0.56 |
| | 63 | 71,239<br>1,449 | 0.18 | 80,855<br>1,342 | 0.21 | 67,315<br>1,424 | 0.47 |
| 2 | 30 | 191,498<br>1,134 | 0.23 | — | — | 176,998<br>1,165 | 0.68 |
| | 42 | 136,200<br>820 | 0.15 | — | — | 132,000<br>695 | 0.44 |
| 3 | 25 | 437,694<br>1,190 | 0.36 | — | — | 410,140<br>1,100 | 0.81 |
| | 51 | 114,670<br>715 | 0.23 | 126,411<br>670 | 0.24 | 110,948<br>678 | 0.59 |
| | 58 | 74,187<br>658 | 0.20 | 83,333<br>785 | 0.18 | 72,452<br>587 | 0.42 |

* SA = dpm/mg.

TABLE X

*Conversions of β-Sitosterol-$^3$H and Cholesterol-$^{14}$C into Bile Acids after Pulse Labeling Intravenously*

| Time after pulse labeling | Plasma | | Cholic acid | | Deoxy and chenodeoxycholic acids | |
|---|---|---|---|---|---|---|
| | $^3$H | $^{14}$C | $^3$H | $^{14}$C | $^3$H | $^{14}$C |
| ys | *dpm/mg of total neutral sterols* | | *dpm/mg bile acids* | | *dpm/mg bile acids* | |
| 7 | 1220 | 3022 | 1140 | 3316 | 900 | 3000 |
| 4 | 871 | 2486 | 990 | 2680 | 865 | 2552 |
| 1 | 565 | 2106 | 660 | 1960 | 745 | 1800 |
| 9 | 464 | 1889 | 518 | 1610 | 502 | 1550 |
| 6 | 333 | 1607 | 426 | 1500 | 478 | 1420 |
| 3 | 265 | 1453 | — | — | 364 | 1120 |
| 2 | 127 | 820 | 212 | 645 | 127 | 550 |
| 8 | 429 | 1190 | 270 | 1016 | 426 | 1450 |
| | 171 | 715 | 250 | 730 | 178 | 634 |

## DISCUSSION

*Daily turnover of β-sitosterol by two methods.* In the present studies the turnover of β-sitosterol was investigated in four patients by two independent methods. Analysis of the decay curve of plasma specific activity of β-sitosterol after pulse labeling intravenously indicated that the turnover of β-sitosterol conforms to a two pool model, just as in the case of cholesterol. According to the theory of the two pool model (12) the daily production rate of β-sitosterol ($PR_A$) should equal the amount of new β-sitosterol which enters the more rapidly exchangeable pool (pool A, Fig. 4) exclusive of recirculated sterol. In five studies of four patients the rate of turnover calculated in this manner varied from 6 to 14 mg/day. In the same patients turnover data were obtained independently by application of the isotopic balance method (11): here, the daily turnover of β-sitosterol is equal to the sum of the excretion of radioactivity

the fecal neutral and acidic steroids derived from sitosterol-$^3$H administered intravenously, divided by the specific activity of plasma β-sitosterol. Values for the total daily fecal excretion of neutral and acidic steroids derived by the latter method were almost identical with values for turnover calculated by kinetic analysis of the two pool model. This close agreement of data obtained by two independent methods appears to validate the assumptions on which both techniques are based.

*Evidence for lack of endogenous synthesis.* We have presented four lines of evidence that β-sitosterol was not synthesized in our patients. (*a*) In five patients fed diets free of β-sitosterol, the fecal neutral steroid fraction was free of β-sitosterol, 24-ethyl coprostanol, and 24-ethyl coprostanone. We use the term "free" operationally, in the sense that the sensitivity of the analytical methods used in the present work sets an upper concentration limit of 65 ng of β-sitosterol per ml of plasma or per g of homogenate. (*b*) When patient 1*a* was fed sitosterol labeled with β-sitosterol-$^3$H of constant specific activity, plasma β-sitosterol specific activity reached the same level as that of the sterol fed. If β-sitosterol had been synthesized endogenously, the specific activity of plasma β-sitosterol would have been lower than that of the fed sterol, reflecting dilution by nonradioactive β-sitosterol produced endogenously. (*c*) In this patient the specific activity of the fecal neutral steroids derived from β-sitosterol was identical with that of the sterol fed. This correspondence would not have been obtained, had endogenous synthesis of β-sitosterol occurred in the intestinal mucosa. (*d*) In five studies a linear relationship was noted between the amount of β-sitosterol absorbed per day and that retained in the body; this line extrapolated to the zero intersect.

*Turnover = absorption.* Since all our evidence confirmed the widely held belief that β-sitosterol is not synthesized endogenously in man, the only entry into pool A must have occurred through absorption of exogenous β-sitosterol. Therefore, daily turnover of β-sitosterol in the steady state, whether calculated by analysis of the two-pool model or through application of the isotopic balance method, must equal daily absorption. Table VI shows that 6–14 mg of β-sitosterol was absorbed each day, or 0.6–5.2% of the daily intake. These very low values for percentage absorption directly confirm the previous estimates of Gould (3, 4) and of Borgström (5).

*β-Sitosterol as internal standard for sterol balance studies.* Previous investigators (6–9) attempted to determine the absorption of β-sitosterol in rats by the method of sterol balance and reached very different conclusions from those presented here. They ascribed to absorption the differences between intake and fecal output; these balance differences ranged from 22 to 53%. But it is our belief that losses of this magnitude were more likely due to degradation of the sterol ring structure during intestinal transit (10). In the present study in man, two patients (2 and 3) showed appreciable losses of nonradioactive dietary β-sitosterol by the sterol balance method (35 and 37%, respectively), while in a third patient (1*b*) the entire intake of dietary β-sitosterol was recovered in the feces. Despite these large differences in recovery that we have ascribed (10) to varying degrees of degradation of the sterol ring structure in different patients, the calculations of daily absorption from kinetic analysis of the two pool model were remarkably similar in all three patients. Therefore, we have concluded that the absorption of β-sitosterol cannot be accurately measured by subtracting fecal output from dietary intake. Indeed, all of the findings in the present study reinforce our reliance on the use of nonradioactive dietary β-sitosterol as an internal standard in sterol balance studies to correct for losses of cholesterol during intestinal transit: first, endogenous synthesis of β-sitosterol apparently does not occur in man, and second, its absorption in the human intestine is quantitatively so small (< 5% of intake) that it meets all the criteria of an ideal internal standard for balance studies on sterols (10).

*Differences in metabolism of cholesterol and β-sitosterol.* The present data emphasize certain differences in the metabolism of β-sitosterol ($C_{29}$) which is structurally similar to cholesterol ($C_{27}$) but contains an additional ethyl group at C-24. We have confirmed that this plant sterol is absorbed from the intestine only about one-tenth as effectively as cholesterol and that it is secreted into the bile more rapidly than cholesterol. It also appears to be esterified more slowly than cholesterol and it is also distributed differently between the two theoretical body pools. On the other hand, β-sitosterol is converted as rapidly as cholesterol into the same primary bile acids.

The mechanisms by which the two sterols are distinguished at the level of the intestinal mucosal cell remain unknown. In the rat, Sylvén and Borgström (26) have noted that the ratio of the two sterols in the intestinal lymphatics is the same as that in the mucosal cells of the intestine; hence, they concluded that the difference in absorption was not due to differences in any intracellular process nor in the transport of these sterols into the intestinal lymphatics. Rather, it seemed to them more likely that the distinction is made earlier in the absorptive process, either by a lesser micellar solubilization of plant sterol or by a slower transport through the outer surface of the mucosal cell. It seems to us, however, that the rate of esterification of β-sitosterol may be the factor limiting its absorption. Swell, Trout,

Field, and Treadwell (9) found that, while β-sitosterol, like cholesterol, is taken up by the intestinal wall, cholesterol is largely esterified before incorporation into chylomicrons. β-Sitosterol, on the other hand, is apparently not esterified in the intestinal wall: Kuksis and Huang (27), studying dogs with thoracic duct fistulas, observed that virtually all of the plant sterols in chyle are unesterified, whereas most of the cholesterol in chyle is esterified.

Two factors appear to contribute to the more rapid fractional turnover rate of β-sitosterol as compared with that of cholesterol. First, as discussed above, in passage through the small intestine at least 10 times more cholesterol than β-sitosterol is returned into body pools through reabsorption. Secondly, it seems clear from the results of the present study that mechanisms exist in the liver whereby β-sitosterol is preferentially selected for more rapid secretion into the bile as neutral sterol. The mechanisms by which the liver cell distinguishes between β-sitosterol and cholesterol are just as unclear as in the case of the intestinal cell. Again, we can visualize the possibility that a retardation in esterification of β-sitosterol, compared to that of cholesterol might make the free sterol more available for secretion into bile, but other possibilities should be considered. The intracellular routes of travel of the two sterols through the liver cell from plasma to biliary canaliculus are still not known; nor do we know whether the sterols in the endoplasmic reticulum are in equilibrium with those in the mitochondria or cell sap, nor how inconstant the sterol contents of the different membranous structures may be. At any rate, we have shown for the first time in any species that β-sitosterol is converted into the same primary bile acids as cholesterol, i.e., cholic acid and chenodeoxycholic acid. The similarities in isotope ratios in Table X suggest that the enzymes responsible for this conversion fail to differentiate one sterol from the other. At what stage the ethyl group at C-24 is removed remains to be determined. Several species of phytophagous insects are known to dealkylate $C_{29}$ and $C_{30}$ sterols to provide cholesterol during their larval growth (28). Therefore, it would be of considerable importance to ascertain whether the β-sitosterol incorporated into bile acids was initially dealkylated to cholesterol, or whether dealkylation occurred simultaneously with side chain oxidation during bile acid synthesis.

Striking differences in distribution of the two sterols in the two theoretical pools of readily exchangeable sterols (pools A and B) have been demonstrated in this study. According to theory (12, 24) the two pool model permits the calculation of the sizes of pools A and B. For cholesterol the size of pool A can be calculated from data obtained from specific activity-time curves of plasma cholesterol; but the size of pool B can be determined only if entry and removal of cholesterol from this pool are negligible (except for isotopic exchange with pool A). If one makes the latter assumption, the data of Goodman and associates indicate that in six adult normocholesterolemic men and women pool A varied from 15 to 27 g; pool B from 27 to 45 g. Our own data (similarly derived) in 21 adult patients with hyperlipoproteinemic states showed that pool A varied from 16 to 32 g, pool B from 21 to 49 g; the lack of difference between normo- and hypercholesterolemic patients is astonishing, if true. In contrast, the present study shows that, for β-sitosterol pool A was invariably the larger pool, ranging in hypercholesterolemic adults from 52 to 125 mg, with only 21 to 76 mg in pool B. Thus, in all adults tested, cholesterol is more richly distributed in pool B than in pool A; the converse holds for β-sitosterol. Thus, the total amount of readily exchangeable cholesterol is vastly greater than for β-sitosterol and furthermore the distribution of the two sterols in the body is different. Why this difference exists remains to be shown, but it seems to rule out the possibility of using the distribution of the plant sterol as a guide to cholesterol distribution in specific tissue pools.

## ACKNOWLEDGMENTS

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# Tall Oil Fatty Acids in the Diet of Growing Rats*

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From tall oil fatty acid distillate it is possible to prepare ethyl and glyceryl esters, which in their physical and chemical properties resemble edible oils, *e.g.*, soybean oil,[1-4] in many respects. The use of these esters as animal fodder ingredients and the results of different feeding experiments have been presented by M. Antila *et al.*[5] and V. Antila *et al.*[6-7]

Experiments have now been conducted to explore the possibilities of using tall oil fatty acid ethyl and glyceryl esters as food fat. The fats and oils used were obtained from the Raisio Factories' Central Laboratory.

Weanling male rats of the Sprague-Dawley strain weighing approximately 40—50 grams each were used in the experiments. The animals were divided into groups of ten and kept in individual wire-bottom cages. Their weights and food intakes were recorded regularly. The animals were given food and water *ad libitum*. The fat to be studied was added in relatively high amounts so that it supplied either 30 or 60 % of the total calories (corresponding to 13.5 and 35.0 wt.-%) to the basic diet which consisted of graham flour, casein, dried brewer's yeast, and salt mixture. Corresponding amounts of soybean oil, butter, or margarine were usually added to the diets of the control groups. The 60 cal.-% level can be consid credalmost unphysiological for rats, but it was used for practical reasons; the effects on the growth are quite rapid and strong at this level. Most of the experiments lasted 3 weeks.

When the animals received 60 % of their calories in the form of the unrefined ethyl esters of tall oil fatty acids, their growth stopped completely and the whole group died within 10 days. The animals receiving refined ethyl esters of tall oil fatty acid distillate also grew rather slowly in comparison with the group fed a corresponding quantity of soybean oil ethyl ester, and about half of the animals died during the experiment (Fig. 1).

The effect of refined glyceryl esters of tall oil fatty acids did not differ much from that of the ethyl esters; the growth of the animals receiving 60 % of their calories as glyceryl esters was extremely slow as compared to the group which was given a corresponding amount of soybean oil. At the 30 cal.-% level the growth of the group receiving glyceryl esters was already better, but it was still markedly poorer compared to the control group

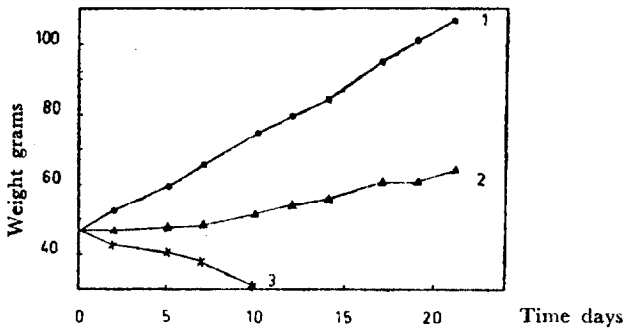


Fig. 1. Average body weights of rats fed 30 % of the calories from ethyl ester of soybean oil (1), refined ethyl esters of tall oil fatty acids (2) or unrefined ethyl esters of tall oil fatty acids (3).

* A paper presented at a Symposium on Fats and Oils held by the Finnish Chemical Society on October 9 th, 1963.

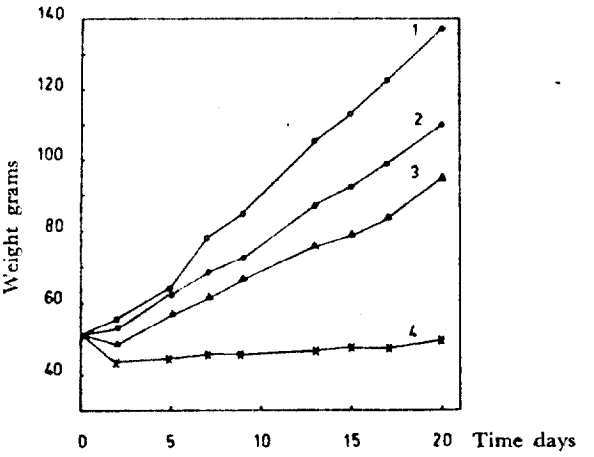


Fig. 2. Average body weights of rats fed 30 or 60 % of the calories from soybean oil (1 and 2) and 30 or 60 % of the calories from glyceryl esters of tall oil fatty acids (3 and 4).

on a diet containing 30 % of the calories as soybean oil (Fig. 2). Both ethyl and glyceryl esters of tall oil fatty acids evidently contain a factor or factors which retard the growth of animals; the effect appears particularly clearly at the 60 cal.-% level. The unrefined ethyl esters are even toxic.

Hydrogenation of the tall oil glyceryl ester considerably improved its growth effect. When a diet in which 30 % of the total calories were furnished by this hydrogenated glyceryl ester was fed to experimental animals, their growth was almost comparable to that of control groups receiving hydrogenated and unhydrogenated soybean oil (Fig. 3).

According to Aho *et al.*[8], Lehtinen *et al.*[9] and Elomaa *et al.*[10] the fatty acid distillate of Finnish tall oil contains about 10 % *cis*-5,9,12-octadecatrienoic acid. When a 75 % concentrate of this acid, obtained by countercurrent extraction, was esterified with ethanol and added at the 30 cal.-% level to the diet of rats their growth at first completely stopped. Gradually the weight of the animals started to rise, but the rate of growth remained low as compared with the growth of rats on diets containing linseed or tall oil ethyl esters. (Fig. 4). These results together with those obtained with the

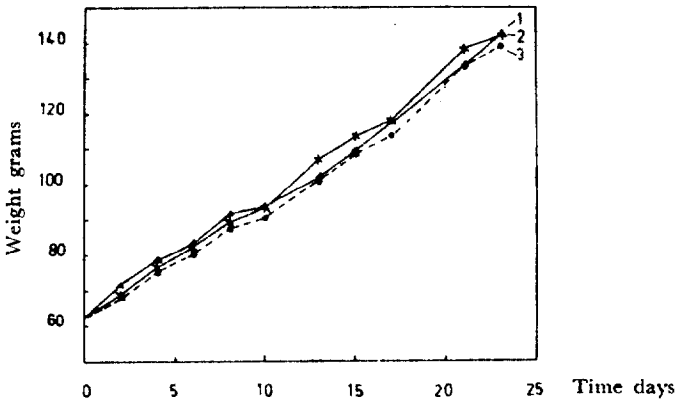


Fig. 3. Average body weights of rats fed 30 % of the calories from hydrogenated soybean oil (1), soybean oil (2) or hydrogenated glyceryl esters of tall oil fatty acids (3).

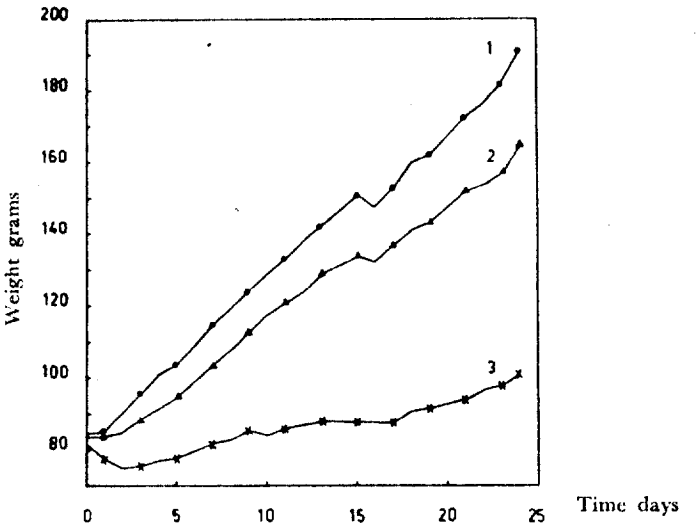


Fig. 4. Average body weights of rats fed 30 % of the calories from ethyl ester of linseed oil (1), ethyl esters of tall oil fatty acids (2) or ethyl ester of *cis*-5,9,12-octadecatrienoic acid (3).

hydrogenated glyceryl ester referred to above indicate that the growth-retarding factor is associated with polyunsaturated fatty acids and with *cis* -5,9,12-octadecatrienoic acid in particular. This acid itself, however, is not "guilty" since an extract of pine seeds known to contain about 20 % of this acid[10], showed no growth-retarding effect.

In addition to the short-term growth experiments reported here, a long-term growth experiment lasting 2 ½ months was undertaken, and it was followed by a reproduction experiment. Both male and female rats were used in the experiments, and they were given diets containing 30 and 60 cal.-% levels of a fat mixture of the margarine type which was prepared by interesterification of hydrogenated tall oil glyceride and hydro-

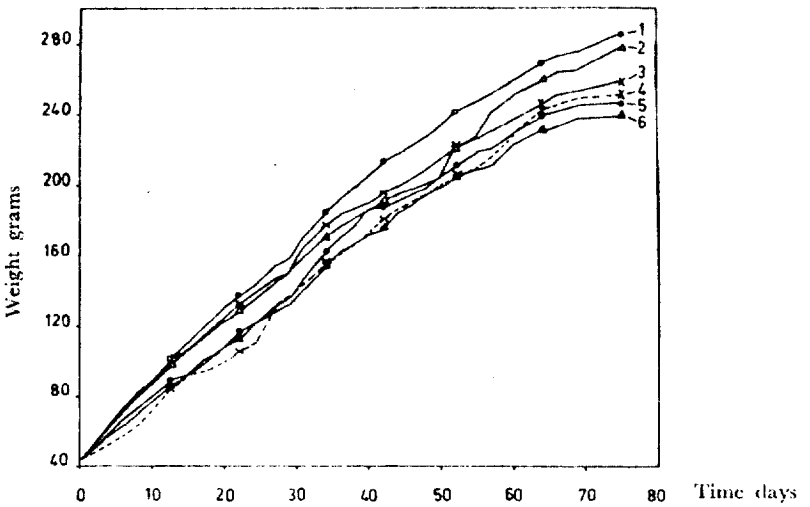


Fig. 5. Average body weights of male rats fed 30 % of the calories from butter (1), »tall oil margarine» (2) or margarine (3) and 60 % of the calories from margarine (4), butter (5) or »tall oil margarine» (6).

genated soybean oil. The control groups were given corresponding amounts of commercial butter and margarine. The animals receiving the »tall oil margarine» at the 30 cal.-% level grew very well. Of the male rats, the control group receiving butter grew at the highest rate, but the »tall oil margarine» group did not fall far behind it. At the 60 cal.-% level the animals on the »tall oil margarine» diet grew at a slightly lower rate than the controls, although no statistically significant differences could be detected (Fig. 5). Of the female rats, the growth rate of the group receiving 30 % of the calories as »tall oil margarine» was even higher than that of the control groups, but at the 60 cal.-% level the females were also observed to fall behind in growth in comparison with the other groups; the difference was very small, however, and not statistically significant.

In the reproduction experiments the »tall oil margarine» groups, both the 30 and the 60 cal.-% level groups, gave birth to litters of normal size and the females were able to wean them normally.

In histopathological investigations no pathological changes could be detected in the hearts, livers, kidneys and thyroid glands of the animals in this experiment.

On the basis of the results of these growth experiments with rats, it seems evident that refined tall oil glyceryl esters can, after certain processes such as hydrogenation and interesterification, be used at least in small amounts in the diets of experimental animals without endangering their health or growth. Investigations are being continued with the aim of identifying the growth-retarding factors and studying the physiological effects of tall oil fatty acid preparations.


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# Range-Finding Toxicity Data: List VI

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A table presents acute toxicity and irritation data on more than 300 compounds, accumulated in a continuing program for screening potential commercial products. The standardized experimental methods are described.

DURING the seven years since the appearance of our last communication on the range-finding test,[1] many additional data have become available for presentation. The following Table lists these data under names conforming to the nomenclature used in *Chemical Abstracts*. This may make them unrecognizable to those who know them only by their trade names. For example: ethers of ethylene glycol are sold as CELLOSOLVE solvents, of diethylene glycol as CARBITOL solvents, and of propylene and dipropylene glycol as UCAR solvents. All materials included here are either in commercial production or have been evaluated for commercial potential within the past few years.

As has been stated in earlier communications,[1-5] the range-finding test is relied upon only to allow predictions of the comparative hazards of handling new chemicals. Acute toxicity studies, no matter how precisely executed, yield no more than an indication of the degree of care necessary to protect exposed workman or lead to an opinion that certain technically feasible applications of a chemical may or may not eventually be proved safe.

Our last paper[1] included data for epoxyethylbenzene. Recently obtained data upon another sample differ somewhat from that previously published. The latest assay yields a single-oral $LD_{50}$ value of 2.83 ml/kg. Four hours in a concentrated vapor atmosphere caused no deaths among six rats while 1000 ppm inhaled for a similar period of time killed only two of six rats.

Because of minor changes in experimental methods and to eliminate reference to earlier papers in this series,[1-5] the methods for obtaining the range-finding data are briefly described.

Single oral dose toxicity is estimated by the gastric intubation of groups of five nonfasted, Carworth-Wistar male rats, or in rare instances indicated in the Table of female rats, four to five weeks of age and 90 to 120 grams in weight which have been reared in our own colony and maintained from time of weaning on Rockland rat diet, complete. The dosages are arranged in a logarithmic series differing by a factor of two. Whenever possible, the chemical is administered undiluted. When a lesser concentration is necessary, solution in water or corn oil or suspension in semi-solid agar are the preferred expedients. Occasionally, a 1% solution of TERGITOL Penetrant 7 (essentially an aqueous solution of 25% sodium 3,9-diethyl-6-tridecanol sulfate) has been used as a dispersing agent. Based upon mortalities during a 14-day observation period, the most probable $LD_{50}$ value and its fiducial range are estimated by the method of Thompson[6] using the Tables of Weil.[7] The figures in parentheses show limits of $\pm$ 1.96 standard deviations while the absence of parentheses indicates that no range is calculable because no dosage resulted in fractional mortality.

Penetration of rabbit skin is estimated by a technique closely akin to the one-day cuff method of Draize and associates,[8] using groups of four male albino New Zealand rabbits weighing 2.5 to 3.5 kg. The fur is removed from the entire trunk by clipping, and the dose is retained beneath an impervious plastic film. Dosages greater than 20 ml/kg cannot be retained in contact with the skin. The animals are immobilized during the

24-hour contact period, after which the film is removed and the rabbits are caged for the subsequent 14-day observation period. The $LD_{50}$ is calculated as described above.

Concentrated vapor inhalation consists of subjecting groups of six male or female albino rats to a flowing stream of vapor-ladened air. The vapor-air mixture is generated by passing 2.5 liters/minute of dried air at room temperature through a fritted glass disc immersed to a depth of at least one inch in approximately 50 ml of the test chemical contained in a gas-washing bottle. Inhalations are continued for time periods in a logarithmic series with a ratio of two extending from one-fourth to eight hours, until the inhalation period killing about half the number of rats within 14 days is defined. For inhalation periods of ten, five and two minutes in duration, a static technique is used whereby 50 to 100 grams of material, spread over a shallow tray 200 square inches in area, is placed in a 120-liter sealed chamber for at least 24 hours. Six rats are then rapidly introduced by means of a drawer-type cage designed to minimize vapor loss. This static technique, continuing for a maximum of eight hours if necessary, is also employed for mixtures of liquids and for solids. The Table records the longest inhalation period which permitted all rats to survive the two-week observation period. A symbol is used to indicate those few instances when the shortest period attempted caused 100% mortality.

Inhalation of metered vapor concentrations by rats is conducted with flowing streams of vapor prepared by various styles of proportioning pumps.[9,10] Inhalation periods are usually of four hours' duration unless slight toxicity enforces an eight-hour period. Concentrations recorded are nominal and not analytically verified. They are in an essentially logarithmic series with a factor of two, and the Table records the concentration yielding fractional mortality among six rats within 14 days. Where no fractional mortality was observed, usually both the concentration yielding no mortality and that yielding complete mortality are indicated.

Primary skin irritation on rabbits is recorded in a 10-grade ordinal series and is based upon the severest reaction that develops on the clipped skin of each of five albino rabbits within 24 hours of the uncovered application of 0.01 ml of undiluted sample or of solutions in water, propylene glycol, or acetone. Grade 1 in the Table indicates no irritation and Grade 2 the least visible capillary injection from the undiluted chemical. Grade 6 indicates necrosis when undiluted and Grade 10 indicates necrosis from a 0.01% solution.

Eye injury in rabbits is recorded in a 10-grade ordinal series and is based upon the degree of corneal necrosis that results from instillation of various volumes and concentrations of chemical, as detailed by Carpenter and Smyth.[11] Grade 1 in the Table indicates at most a very small area of necrosis resulting from 0.5 ml of undiluted chemical in the eye. Grade 5 indicates a so-called severe burn from 0.005 ml, and Grade 10 indicates a severe burn from 0.5 ml of a 1% solution in water or propylene glycol.

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RANGE-FINDING TOXICITY DATA

| Material Studied | Single Oral $LD_{50}$ for Rats Ml/Kg | Single Skin Penetration $LD_{50}$ for Rabbits Ml/Kg | Concentrated Vapor Inhalation by Rats Maximum for No Death | Inhalation of Metered Vapor Concentration by Rats: Concentration ppm | Time, hr. | Mortality | Irritation on Uncovered Rabbit Belly | Corneal Injury in Rabbits |
|---|---|---|---|---|---|---|---|---|
| **Hydrocarbons** | | | | | | | | |
| Benzene | 11.4 (8.4-16.9) | — | 5 min. | 16,000 | 4 | 4/6 | 3 | 3 |
| Diethylcyclohexane (mixed isomers) | 64 | 2.5 | 1 hr. | 2,000 | 4 | 2/6 | 4 | 1 |
| Ethylbenzene | 5.46 (5.09-5.86)* | 17.8 | 1 hr. | 4,000† | 4 | 3/6 | 4 | 2 |
| Fluoranthene | 2.00 (1.27-3.13)* | 3.18 (2.35-4.29)* | 8 hr. | — | — | — | — | 7 |
| 3a,4,7,7a-Tetrahydro-4, 7-methanoindene (Dicyclopentadiene) | 0.41 (0.31-0.53) | 4.46 (2.44-8.15) | 30 min. | 500† | 4 | 1/6 | 5 | 2 |
| m-Xylene | 7.71 (6.24-9.53) | 14.1 | 2 hr. | 8,000 | 4 | 10/12 | 5 | 1 |
| **Hydroxy Compounds** | | | | | | | | |
| 2-Butene-1-ol | 0.93 (0.67-1.30) | 1.27 (0.94-1.72) | 15 min. | 1,000† | 4 | 0/6 | 1 | 7 |
| Cyclohexanol | 2.06 (1.95-2.18)* | — | 8 hr. | — | — | — | 2 | 7 |
| 2-Cyclopentene-1-ol | 0.47 (0.34-0.66) | 0.18 | 1 hr. | 500† | 4 | 0/6 | 2 | 5 |
| 2,2-Dimethyl-1,3-butanediol | 10.0 (6.38-15.7) | >10 | 8 hr. | — | — | — | 1 | 5 |
| 2,2-Dimethylbutanol | 2.33 (1.74-3.12) | 1.77 (1.09-2.86) | — | — | — | — | 2 | 7 |
| 2,5-Dimethyl-1,2,6-hexanetriol | 24.2 (19.7-30.1)* | >20 | — | — | — | — | 1 | 2 |
| 2,3-Dimethylpentanol | 2.38 (1.46-3.87) | 2.5 (1.7-3.7) | 8 hr. | — | — | — | 2 | 9 |
| Dodecylphenol (mixed isomers) | 2.14 (1.54-2.99) | 5.00 (3.38-7.40) | — | — | — | — | 7 | 2 |
| 1-Ethynylcyclohexanol | 0.69 (0.43-0.84) | 1.00 (0.68-1.48) | 8 hr. | — | — | — | 3 | 8 |
| 2-Ethyl-4-methylpentanol | 4.29 (3.07-5.98) | >5.0 | 8 hr. | — | — | — | 3 | 5 |
| Heptadecanol (mixed primary isomers) | 51.6 (44.4-59.9) | 16.8 (12.0-23.6) | 8 hr. | — | — | — | 3 | 1 |
| 4-Hexene-1-yne-3-ol | 0.034 (0.024-0.047)* | 0.071 | 1 hr.§ | 62.5† | 4 | 1/6 | 2 | 7 |
| 2-Hydroxymethyl-norcamphane | 1.68 (1.13-2.39) | 0.71 | 8 hr. | — | — | — | 3 | 5 |
| 3-Hydroxymethyltetrahydropyran, (mixed 2,4- and 3,4-dimethyl isomers) | 3.10 (2.69-3.57)* | — | 8 hr. | — | — | — | 1 | 5 |
| 5-Indanol | 3.25 (2.48-4.26)* | 0.45 (0.24-0.82)* | — | — | — | — | 7 | 9 |
| 2-Methylbutanol | 4.92 (3.75-6.46) | 3.54 | 8 hr. | — | — | — | 2 | 8 |
| Methylheptanol (mixed primary isomers) | 5.16 (4.17-6.38) | 2.52 (1.86-3.41) | 4 hr. | — | — | — | 2 | 5 |
| 4-Methylpentanol | 6.50 (4.23-9.99) | 3.97 (2.93-5.37) | 8 hr. | — | — | — | 2 | 5 |
| 1-Naphthol | 2.59 (2.10-3.21)* | 0.88 (0.33-2.35)* | 8 hr. | — | — | — | — | 9 |
| 1,5-Pentanediol | 5.89 (5.38-6.44)* | >20 | 8 hr. | — | — | — | 1 | 2 |
| Tert-Pentylphenol | 3.08 (2.49-3.81)* | 2.00 (1.35-2.96)* | 8 hr. | — | — | — | 6 | 10 |
| 2-Propylheptanol | 6.73 (4.79-9.45) | >10 | 8 hr. | — | — | — | 2 | 2 |
| Undecanol (mixed primary isomers) | 17.2 (12.3-23.9) | 7.07 (2.33-21.4) | 8 hr. | — | — | — | 4 | 2 |
| 2,2,4-Trimethyl-1,3-pentanediol | 3.73 (2.68-5.21)* | 6.30 (4.66-8.52) | — | — | — | — | 2 | 7 |
| 2,2,4-Trimethylpentanol | 3.73 (2.68-5.21) | 6.30 (4.66-8.52) | 8 hr. | — | — | — | 2 | 7 |
| **Ethers** | | | | | | | | |
| Allyl vinyl ether | 0.55 (0.30-1.02)* | — | 5 min.§ | 8,000 | 4 | 2/6 | 1 | 2 |

RANGE-FINDING TOXICITY DATA—CONTINUED

| Material Studied | Single Oral $LD_{50}$ for Rats Ml/Kg | Single Skin Penetration $LD_{50}$ for Rabbits Ml/Kg | Concentrated Vapor Inhalation by Rats Maximum for No Death | Inhalation of Metered Vapor Concentration by Rats: Concentration ppm | Time, hr. | Mortality | Irritation on Uncovered Rabbit Belly | Corneal Injury in Rabbits |
|---|---|---|---|---|---|---|---|---|
| 2,3-Bis-[2,3-epoxypropoxyl-1,4-dioxane | 1.07 (0.77-1.50)* | 1.59 (1.17-2.20) | 8 hr. | — | — | — | 4 | 7 |
| Cyclopentyl ether | 0.47 (0.34-0.66) | 1.41 | 2 hr. | 250 | 4 | 5/6 | 4 | 2 |
| 1,3-Dimethoxybutane | 3.73 (2.68-5.21) | 10.0 (4.6-21.9) | 1 hr. | 8,000 | 4 | 4/6 | 2 | 2 |
| 4,4-Dimethyl-1,3-dioxane | 3.73 (2.68-5.21) | 3.54 | 4 hr.§ | 8,000† | 4 | 2/6 | 2 | 7 |
| 2-Ethoxy-3,4-dihydro-1,2-pyran | 6.16 (5.52-6.88)* | 3.56 | — | 8,000† | 4 | 1/6 | 3 | 1 |
| 2-Ethoxy-4-methyl-3,4-dihydropyran | 5.63 | 1.34 (0.96-1.87) | 2 hr. | — | — | — | 3 | 2 |
| Ethyl ether | 3.56 (3.23-3.92)* | >20 | 5 min. | 32,000† | 4 | 3/6 | 1 | 2 |
| 2-Ethyl-2-methyl-1,3-dioxolane | 2.83 | 10.0 (4.6-21.9) | — | 4,000 | 4 | 4/6 | 2 | 2 |
| Ethyl-1-propenyl ether | 19.0 (13.4-27.0) | — | 5 min. | 8,000 | 4 | 0/6 | 2 | 2 |
| Isobutyl vinyl ether | 17.0 (12.2-23.8) | 20 | 10 min.§ | 16,000 | 4 | 3/6 | 1 | 2 |
| 1-Methoxy-1,3-butadiene | 2.14 (1.54-2.99) | — | 15 min.§ | — | — | — | 4 | 2 |
| 4,4'-Methylenediphenol | 4.95 (3.82-5.52)* | — | 8 hr. | — | — | — | — | 9 |
| 4-Methyl-2-vinyl-1,3-dioxolane | 0.89* | 0.79 (0.59-1.07) | — | 1,000 | 4 | 3/6 | 2 | 4 |
| 1-Propenyl-2-butene-1-yl ether | 8.00 (5.10-12.5) | >10 | 1 hr. | 5,000† | 4 | 2/6 | 4 | 2 |
| 1,1,3-Triethoxybutane | 4.92 (3.75-5.46) | 1.77 | 2 hr | 2,000 | 4 | 5/6 | 2 | 8 |
| 1,1,3-Triethoxyhexane | 17.0 (12.2-23.8) | — | 4 hr. | — | — | — | 2 | 2 |
| **Hydroxy Ethers** | | | | | | | | |
| Diethylene glycol divinyl ether | 3.73 (2.68-5.21)* | 14.1 | 4 hr. | — | — | — | 2 | 1 |
| Diethylene glycol ethyl methyl ether | 6.50 (4.95-8.53) | 7.07 | 8 hr. | — | — | — | 2 | 4 |
| Diethylene glycol mono-2-methylpentyl ether | 5.66 | 1.58 (1.16-2.13) | 8 hr. | — | — | — | 3 | 6 |
| Diethylene glycol ethyl vinyl ether | 11.3 | 8.41 (5.05-14.0 | 8 hr. | — | — | — | 2 | 1 |
| Dipropylene glycol monomethyl ether | 5.66 | 10.0 (6.8-14.8) | 8 hr. | — | — | — | 1 | 2 |
| Ethylene glycol mono-2-methylpentyl ether | 3.73 (2.68-5.21) | 0.44 | 4 hr. | — | — | — | 2 | 6 |
| Ethylene glycol mono-2,6,8-trimethyl-4-nonyl ether | 5.36 (3.84-7.48) | 3.15 (1.85-4.26) | 8 hr. | — | — | — | 2 | 5 |
| Propylene glycol monomethyl ether | 5.66 | 14.1 (8.8-22.9) | 4 hr. | 10,000 | 4 | 0/6 | 2 | 3 |
| Triethylene glycol monobutyl ether | 6.73 (4.13-11.0) | 3.54 (1.06-11.8) | 8 hr. | — | — | — | 3 | 5 |
| Triethylene glycol monomethyl ether | 11.8 | 7.1 | 8 hr. | — | — | — | 2 | 1 |
| **Aldehydes** | | | | | | | | |
| 4-Cyclohexene-1-carboxaldehyde | 2.46 (1.88-3.23) | 1.77 | 4 hr. | 2,000 | 4 | 0/6 | 4 | 8 |
| 1-Decanal (mixed isomers) | 3.73 (2.43-5.74) | 5.04 (3.73-6.82) | 8 hr. | — | — | — | 6 | 1 |
| 2,3-Dimethyl-4-pentenal | 5.66 | >10 | 2 hr. | 10,000 | 4 | 5/6 | 1 | 2 |

RANGE-FINDING TOXICITY DATA—CONTINUED

| Material Studied | Single Oral $LD_{50}$ for Rats Ml/Kg | Single Skin Penetration $LD_{50}$ for Rabbits Ml/Kg | Concentrated Vapor Inhalation by Rats Maximum for No Death | Inhalation of Metered Vapor Concentration by Rats: Concentration ppm | Time, hr. | Mortality | Irritation on Uncovered Rabbit Belly | Corneal Injury in Rabbits |
|---|---|---|---|---|---|---|---|---|
| 2,3-Dimethyl valeraldehyde | 3.54 (2.19-5.72) | 7.1 (4.4-11.4) | 2 hr. | 6,000 | 4 | 0/6 | 2 | 2 |
| Glutaraldehyde, 25% | 2.38 (1.68-3.38) | 2.56 (1.88-3.44) | 8 hr. | — | — | — | — | 9 |
| Glyoxal 29.2% | 7.46 (5.35-10.4) | >20 | 8 hr. | — | — | — | 2 | 3 |
| 4-Hexanal (mixed isomers) | 9.51 (5.84-15.5) | >10 | 4 hr. | — | — | — | 2 | 5 |
| 3-Methoxy butyraldehyde | 0.54 (0.35-0.82) | 0.31 (0.12-0.78) | 15 min. | 500† | 4 | 0/6 | 2 | 8 |
| 2-Methyl-4-cyclohexene-1-carboxaldehyde | 5.66 | 3.15 (2.33-4.26) | 8 hr. | — | — | — | 5 | 5 |
| 4-Methyl valeraldehyde | 5.66 | 4.46 (2.44-8.15) | 30 min. | 8,000† | 4 | 0/6 | 2 | 3 |
| 4-Octanal (mixed isomers) | 5.63 | 6.35 (4.70-8.59) | 8 hr. | — | — | — | 4 | 2 |
| 4-Pentanal (mixed isomers) | 4.76 (3.35-6.75) | >20 | 5 min. | 8,000 | 4 | 3/6 | 2 | 2 |
| 4-Pentenal | 0.62 (0.40-0.95)*,** | 1.59 (0.71-3.53) | 5 min. | 250 | 4 | 1/12 | 1 | 5 |
| **Ketones** | | | | | | | | |
| Acetone | 10.7 (7.7-15.0)** | >20 | 30 min. | 16,000† | 4 | 1/6 | 1 | 5 |
| 2-Butanone | 6.86 (5.59-8.45)** | >10 | — | 8,000† | 8 | 3/6 | 2 | 5 |
| 2-Heptanone | 1.67 (1.48-1.88)*, ** | 12.6 (9.3-17.0) | 30 min. | 2,000† | 4 | 0/6 | 4 | 2 |
| 4-Hexene-1-yne-3-one | 0.071 (0.044-0.11)* | 0.10 (0.04-0.25) | 5 min.§ | 12.5 | 4 | 2/6 | 2 | 10 |
| 5-Methyl-2-hexanone | 4.76 (2.92-7.75) | 10.0 (6.8-14.8) | — | 2,000† | 4 | 0/6 | 1 | 2 |
| 2-Pentanone | 3.73 (2.68-5.21)* | 8.00 (3.60-17.8) | 30 min. | 2,000† | 4 | 1/6 | 1 | 3 |
| **Acids, Anhydrides, and Lactones** | | | | | | | | |
| Acrylic acid, glacial | 2.59 (2.10-3.21)* | 0.95 (0.67-1.30) | 8 hr. | — | — | — | 6 | 9 |
| Allylsuccinic anhydride | 1.07 (0.77-1.50) | 0.32 (0.23-0.43) | — | — | — | — | 3 | 9 |
| Crotonic anhydride | 2.83 (1.77-4.53)* | — | 8 hr. | — | — | — | 5 | 10 |
| Decanoic acid (mixed isomers) | 3.73 (2.93-4.86) | 1.77 (1.09-2.86) | 8 hr. | — | — | — | 5 | 9 |
| Dimethyl-e-caprolactone (mixed isomers) | 11.3 | 3.54 (2.19-5.72) | 8 hr. | — | — | — | 2 | 5 |
| 2,5-Dimethyl-2-hydroxy-adipaldehyde | 5.89 (5.38-6.44)* | — | 8 hr. | — | — | — | 1 | 7 |
| Glutaric anhydride | 4.46* | 1.78* | — | — | — | — | — | 8 |
| Hexanoic acid (mixed isomers) | 2.05 (1.66-2.53) | 1.05 (0.29-3.85) | 8 hr. | — | — | — | 4 | 9 |
| 2-Hydroxy-3-ethyl-heptanoic acid | 3.40 (3.11-3.72)* | 1.78 | — | — | — | — | 4 | 9 |
| 6-Hydroxytetrahydropyran-2-carboxylic acid lactone | 5.66 | 3.97 (2.93-5.37) | — | — | — | — | 1 | 7 |
| Methyl-e-caprolactone (mixed isomers) | 11.2 | 7.1 | 8 hr. | — | — | — | 2 | 5 |
| 2-Methyl propionic acid | 0.28 (0.17-0.46) | 0.50 (0.37-0.67) | 8 hr. | — | — | — | 6 | 9 |
| Octanoic acid (mixed isomers) | 1.41 (0.88-2.29) | 0.71 | 4 hr. | — | — | — | 5 | 9 |
| Pentanoic acid (mixed isomers) | 1.12 (0.45-2.79) | 0.70 (0.38-1.28) | 8 hr. | — | — | — | 6 | 8 |
| Propionic acid | 4.29 (3.07-5.98)* | 0.56 (0.37-0.67) | 8 hr. | — | — | — | 6 | 9 |

RANGE-FINDING TOXICITY DATA—CONTINUED

| Material Studied | Single Oral $LD_{50}$ for Rats Ml/Kg | Single Skin Penetration $LD_{50}$ for Rabbits Ml/Kg | Concentrated Vapor Inhalation by Rats Maximum for No Death | Inhalation of Metered Vapor Concentration by Rats: Concentration ppm | Time, hr. | Mortality | Irritation on Uncovered Rabbit Belly | Corneal Injury in Rabbits |
|---|---|---|---|---|---|---|---|---|
| Trimethyl-ε-caprolactone (mixed isomers) | 7.43 (5.32-10.4) | 6.30 (4.66-8.52) | 8 hr. | — | — | — | 2 | 5 |
| Esters | | | | | | | | |
| Acetic acid, amyl ester (mixed isomers) | 6.50 (4.95-8.53)* | >20 | 4 hr. | — | — | — | 3 | 2 |
| Acetic acid, 1,3-butadienyl ester | 0.71* | 0.42 (0.30-0.60) | 10 min.§ | 62.5† | 4 | 3/6 | 6 | 9 |
| Acetic acid, 2-butoxyethyl ester | 7.46 (5.35-10.4) | 1.58 (1.16-2.13) | 8 hr. | — | — | — | 1 | 2 |
| Acetic acid, ethyl ester | 11.3** | >20 | 15 min. | 8,000† | 4 | 0/6 | 1 | 2 |
| Acetic acid, isobutyl ester | 15.4 (12.5-19.1) | >20 | 1 hr. | 8,000† | 4 | 4/6 | 1 | 2 |
| Acetic acid, methyl ester | 6.97 (6.32-7.69)* | — | — | 16,000† | 4 | 0/6 | 1 | 5 |
| Acrylic acid, decyl ester | 6.46 (4.93-8.48)* | 6.30 (4.66-8.52) | 4 hr. | — | — | — | 5 | 2 |
| Acrylic acid, tridecyl ester | 44.7 | 6.30 (4.66-8.52) | 8 hr. | — | — | — | 5 | 2 |
| Adipic acid, di-(decyl) ester (mixed isomers) | 20.5 (16.5-25.3) | 8.41 (5.00-14.1) | 8 hr. | — | — | — | 2 | 1 |
| Adipic acid, di-2-propynyl ester | 0.20 (0.13-0.31)* | 0.44 (0.24-0.82) | — | — | — | — | 1 | 2 |
| Azelaic acid, di-(2-ethylhexyl) ester | 8.72 (4.72-16.1) | 20.0 (9.1-43.8) | 8 hr. | — | — | — | 3 | 1 |
| 1,2,4-Butanetricarboxylic acid, tri (2-ethylhexyl) ester | >64 | 20 | — | — | — | — | 1 | 2 |
| Crotylidene dicrotonate | 2.59 (2.10-3.21)* | — | 4 hr. | — | — | — | 4 | 1 |
| Decanoic acid, vinyl ester (mixed isomers) | 6.17 (4.99-7.63) | 14.1 (8.8-22.9) | 8 hr. | — | — | — | 4 | 1 |
| Dibenzoyl diethylene glycol ester | 2.83 (1.93-4.15)* | 20.0 (13.5-29.6) | 8 hr. | — | — | — | 1 | 1 |
| Dibenzoyl dipropylene glycol ester | 9.80 (7.47-12.9)* | >10 | — | — | — | — | 1 | 1 |
| Di-(decanoyl) triethylene glycol ester (mixed isomers) | 7.46 (5.20-10.7) | 11.2 (6.1-20.5) | 8 hr. | — | — | — | 3 | 1 |
| 2,5-Endomethylene cyclohexane carboxylic acid, ethyl ester (mixed formyl isomers) | 7.46 (5.35-10.4) | 10 | — | — | — | — | 3 | 2 |
| 2,5-Endomethylene-3-cyclohexene carboxylic acid, ethyl ester | 4.29 (3.07-5.98) | >5 | — | — | — | — | 3 | 2 |
| 2-Ethylhexanoic acid, 2-ethylhexyl ester | 27* | >20 | 8 hr. | — | — | — | 2 | 2 |
| Formic acid, vinyl ester | 2.82 (2.42-3.30)* | 3.17 (1.56-6.44) | 5 min.§ | 1,000† | 4 | 0/6 | 1 | 9 |
| b-Formyl propionic acid, ethyl ester | 9.87 (7.52-13.0) | 7.1 | — | — | — | — | 2 | 5 |
| Fumaric acid, diethyl ester | 1.78 (1.60-1.98)* | — | 8 hr. | — | — | — | — | 2 |
| Fumaric acid, di-isobutyl ester | 8.12 (6.19-10.7) | 7.49 (5.87-10.7) | 8 hr. | — | — | — | 3 | 2 |
| Hexanoic acid, vinyl ester (mixed isomers) | 19.7 (15.0-25.8) | >20 | 1 hr. | 4,000 | 4 | 5/6 | 2 | 1 |
| Isophthalic acid, di-(decyl) ester (mixed isomers) | >64 | >10 | 8 hr. | — | — | — | 1 | 1 |

RANGE-FINDING TOXICITY DATA—CONTINUED

| Material Studied | Single Oral $LD_{50}$ for Rats Ml/Kg | Single Skin Penetration $LD_{50}$ for Rabbits Ml/Kg | Concentrated Vapor Inhalation by Rats Maximum for No Death | Inhalation of Metered Vapor Concentration by Rats | | | Irritation on Uncovered Rabbit Belly | Corneal Injury in Rabbits |
|---|---|---|---|---|---|---|---|---|
| | | | | Concentration ppm | Time, hr. | Mortality | | |
| Isophthalic acid, di-(2-ethylhexyl) ester | 17.3 (9.4-32.0) | 7.94 (5.87-10.7) | 8 hr. | — | — | — | 1 | 1 |
| Maleic acid, dihexyl ester | 7.34 (6.22-8.66)**, * | — | 8 hr. | — | — | — | 3 | 1 |
| Maleic acid, dimethyl ester | 1.41* | 0.53 (0.38-0.75) | 4 hr. | — | — | — | 3 | 3 |
| Maleic acid, dipentyl ester | 4.92 (3.75-6.46) | >10 | 8 hr. | — | — | — | 2 | 1 |
| Mandelic acid, hexyl ester | 17.0 (12.2-23.8)* | 15.4 (13.3-17.8) | — | — | — | — | 3 | 4 |
| Methyl borate | 6.14 (4.96-7.95) | 1.96 (1.47-2.68) | — | — | — | — | 1 | 3 |
| Octanoic acid, vinyl ester (mixed isomers) | 7.46 (4.86-11.5) | >10 | 4 hr. | — | — | — | 2 | 1 |
| Phthalic acid, di-(decyl) ester | >64 | 16.8 (10.0-28.2) | 4 hr. | — | — | — | 2 | 2 |
| Phthalic acid, di-(tridecyl) ester | >64 | >20 | — | — | — | — | 2 | 2 |
| Propionic acid, vinyl ester | 4.76 (3.35-6.75)* | 10.0 (6.8-14.8) | 5 min. | 4,000 | 4 | 4/6 | 2 | 2 |
| **Epoxy Compounds** | | | | | | | | |
| Acetic acid, 3,4-epoxy-6-methylcyclohexylmethyl ester | 9.8 (6.1-12.0) | 7.94 (5.87-10.7) | 8 hr. | — | — | — | 2 | 2 |
| Acrylic acid, 2,3-epoxypropyl ester | 0.21 (0.15-0.30)* | 0.40 (0.18-0.92) | 30 min. | 62.5† | 4 | 0/6 | 7 | 10 |
| Bis-[2,3-epoxycyclopentyl] ether | 2.14 (1.54-2.99) | — | 8 hr. | — | — | — | 1 | 3 |
| 2,2-Bis-[2,2,2-trimethylethyl] oxirane | 6.69 (4.11-10.9) | 14.1 | 8 hr. | — | — | — | 6 | 1 |
| 1,4-Dichloro-2,3-epoxybutane | 0.71* | 2.83 | 2 hr. | — | — | — | 2 | 2 |
| 1,2-8,9-Diepoxylimonene | 5.63* | 1.77 | 8 hr. | — | — | — | 4 | 3 |
| 1,2-7,8-Diepoxyoctane | 1.07 (0.77-1.50) | 0.32 (0.23-0.43) | 4 hr. | — | — | — | 6 | 7 |
| 1,2-Epoxybutane | 1.41 (0.88-2.29) | 2.10 (1.50-2.95) | — | 4,000† | 4 | 1/6 | 1 | 4 |
| 2,3-Epoxybutyric acid, butyl ester | 0.50 (0.32-0.79) | 2.83 | 8 hr. | — | — | — | 1 | 2 |
| 3,4-Epoxycyclohexanecarbonitrile | 1.23 (0.94-1.62) | 0.99 (0.73-1.34) | 8 hr. | — | — | — | 2 | 5 |
| 4,5-Epoxycyclohexane-1,2-dicarboxylic acid, di-(decyl)ester (mixed isomers) | >64 | >20 | 8 hr. | — | — | — | 2 | 2 |
| 4,5-Epoxycyclohexane-1,2-dicarboxylic acid, di-2-ethylhexyl) ester | >64 | >20 | 8 hr. | — | — | — | 2 | 1 |
| 3,4-Epoxy-2,5-endo-methylenecyclohexanecarboxylic acid, ethyl ester | 4.76 (3.28-6.75) | 3.54 | 8 hr. | — | — | — | 2 | 2 |
| 1,2-b-Epoxyethyl-5,6-epoxybenzene | 2.83* | 0.62 (0.25-1.57) | 8 hr. | — | — | — | 5 | 7 |
| 2,3-Epoxy-2-ethylhexanol | 5.05 (4.49-5.68) | 3.15 (2.33-4.26) | 8 hr. | — | — | — | 1 | 5 |
| 3,4-Epoxy-6-methylcyclohexanecarboxylic acid, allyl ester | 0.50 (0.32-0.79) | 2.83 (0.93-8.58) | 8 hr. | — | — | — | 3 | 1 |
| 3,4-Epoxy-6-methylcyclohexanecarboxylic acid, 3,4-epoxy-6-methylcyclohexylmethyl ester | 4.92 (3.75-6.46) | >10 | 8 hr. | — | — | — | 2 | 1 |

RANGE-FINDING TOXICITY DATA—CONTINUED

| Material Studied | Single Oral $LD_{50}$ for Rats Ml/Kg | Single Skin Penetration $LD_{50}$ for Rabbits Ml/Kg | Concentrated Vapor Inhalation by Rats Maximum for No Death | Inhalation of Metered Vapor Concentration by Rats: Concentration ppm | Time, hr. | Mortality | Irritation on Uncovered Rabbit Belly | Corneal Injury in Rabbits |
|---|---|---|---|---|---|---|---|---|
| 2,3-Epoxypropyl butyl ether | 2.05 (1.56-2.69)* | 2.52 (1.28-4.97) | 2 hr. | 4,000 | 4 | 1/6 | 1 | 5 |
| N-(2,3-Epoxypropyl) diethylamine | 0.42 (0.26-0.68) | 0.79 (0.59-1.07) | 30 min. | 1,000† | 4 | 0/6 | 6 | 9 |
| 9,10-Epoxystearic acid, allyl ester | 1.41 (0.88-2.29) | 15.9 (11.7-21.5) | 8 hr. | — | — | — | 3 | 1 |
| 9,10-Epoxystearic acid, 2-ethylhexyl ester | 30.8 (24.9-38.1) | >20 | 8 hr. | — | — | — | 3 | 1 |
| 1,2-Epoxy-4-vinylcyclohexane | 2.00 (1.28-3.13) | 2.83 | 2 hr. | — | — | — | 2 | 5 |
| Ethylene glycol di-(2,3-epoxy-2-methylpropyl) ether | 7.46 (5.35-10.4) | 3.15 (2.38-4.26) | — | — | — | — | 2 | 5 |
| **Aliphatic and Alicyclic Amines** | | | | | | | | |
| Acrylic acid, N,N-diethylaminoethyl ester | 0.89 (0.56-1.41)* | 0.20 (0.15-0.27) | 2 hr. | — | — | — | 6 | 9 |
| 7-Aminoheptanoic acid, isopropyl ester | 4.00 (2.70-5.92) | 0.89 (0.63-1.25) | 8 hr. | — | — | — | 6 | 9 |
| 3-Aminopropanol | 2.83 | 1.25 (0.84-1.85) | 8 hr. | — | — | — | 5 | 9 |
| Bis-[3-aminopropyl]amine | 0.81 (0.53-1.25) | 0.11 | 8 hr. | — | — | — | 7 | 8 |
| N,N-Bis-[3-aminopropyl] methylamine | 1.54 (1.25-1.91) | 0.14 | 8 hr. | 333 | 1 | 6/6 | 6 | 8 |
| Bis-(2,5-endomethylene-cyclohexylmethyl amine | 1.41* | 0.11 (0.06-0.21) | 8 hr. | — | — | — | 6 | 9 |
| Bis-[a-methylbenzyl] amine | 2.93 (1.52-5.67) | 3.97 (3.60-5.37) | 8 hr. | — | — | — | 6 | 2 |
| N-Butylcyclohexylamine | 0.83 (0.21-0.54)* | 0.53 (0.38-0.75) | 8 hr. | — | — | — | 6 | 9 |
| N-Butyl-a-methyl-benzylamine | 0.36 (0.24-0.52)* | 0.57 (0.35-0.91) | 8 hr. | — | — | — | 4 | 5 |
| Dicylamine | 0.28 (0.14-0.53) | 0.35 (0.22-0.57) | 2 hr. | — | — | — | 6 | 9 |
| Diallylamine | 0.65 (0.49-0.85)* | 0.28 | — | 1,000† | 4 | 0/6 | 6 | 8 |
| 1,8-Diamino-p-menthane | 0.77 (0.62-0.95) | 0.63 (0.47-0.85) | 4 hr. | — | — | — | 7 | 9 |
| Diamylamine | 0.27 (0.19-0.37) | 0.36 | 30 min. | 62.5† | 4 | 4/6 | 6 | 5 |
| 3-(Dibutylamino) propylamine | 0.82 (0.62-1.07)* | 0.27 (0.16-0.45) | 8 hr. | — | — | — | 6 | 9 |
| N,N-Dibutyl-(2-hydroxy-propyl)amine | 1.99 (1.64-2.42)*, ** | — | 8 hr. | — | — | — | 5 | 2 |
| N,N-Dibutylmethylamine | 0.54 (0.38-0.75) | 0.88 | 5 min. | 125† | 4 | 0/6 | 4 | 5 |
| (3-Diethylaminopropyl) amine | 1.41 (1.21-1.65)* | 0.75 (0.54-1.06) | 4 hr. | — | — | — | 6 | 9 |
| Di-(hydroxyethyl)-o-tolylamine | 2.2* | 1.0 | 8 hr. | — | — | — | 1 | 8 |
| (3-Dimethylaminopropyl) amine | 1.87 (1.34-2.60)* | — | 8 hr. | — | — | — | 6 | 9 |
| (1,3-Dimethylcyclo-pentene-1-yl-methyl) amine | 0.81 (0.62-1.07)* | 0.40 (0.30-0.54) | 4 hr. | — | — | — | 6 | 9 |
| Dipropylamine | 0.93 (0.67-1.30)* | 1.25 (0.84-1.85) | 5 min. | 1,000† | 4 | 2/6 | 6 | 9 |
| Di-(tridecyl)amine | 9.85 (7.51-12.9) | 3.54 | 8 hr. | — | — | — | 7 | 1 |

RANGE-FINDING TOXICITY DATA—CONTINUED

| Material Studied | Single Oral $LD_{50}$ for Rats Ml/Kg | Single Skin Penetration $LD_{50}$ for Rabbits Ml/Kg | Concentrated Vapor Inhalation by Rats Maximum for No Death | Inhalation of Metered Vapor Concentration by Rats | | | Irritation on Uncovered Rabbit Belly | Corneal Injury in Rabbits |
|---|---|---|---|---|---|---|---|---|
| | | | | Concentration ppm | Time, hr. | Mortality | | |
| (2,5-Endomethylene-cyclohexylmethyl) amine | 1.41* | 0.52 (0.35-0.76) | 8 hr. | — | — | — | 6 | 9 |
| N-Ethyl(cyclohexyl) amine | 0.59 (0.53-0.67)* | 0.75 (0.54-1.08) | 1 hr. | 500 | 4 | 5/6 | 6 | 9 |
| N-(2-Ethylhexyl)cyclo-hexylamine | 0.71* | 0.11 (0.08-0.15) | 4 hr. | — | — | — | 6 | 5 |
| N-Ethyl(a-methylbenzyl) amine | 0.36* | 0.89 | 4 hr. | — | — | — | 6 | 9 |
| N-(2-Hydroxyethyl) phenylamine | 2.23 (2.01-2.47)* | 0.063 (0.043-0.094) | 8 hr. | — | — | — | 2 | 5 |
| N-Hydroxypropyl-diethylenetriamine | 3.73 (2.68-5.21)* | 1.50 (0.89-2.52) | 8 hr. | — | — | — | 2 | 5 |
| N-(3-Hydroxypropyl)-1, 2-propanediamine | 5.66* | 2.12 (1.51-2.98) | 8 hr. | — | — | — | 6 | 5 |
| N-Methyl(butyl)amine | 0.42 (0.29-0.69)* | 1.26 (0.93-1.70) | 10 min.§ | 2,000 | 4 | 3/6 | 6 | 9 |
| 1,3-Propanediamine | 0.35 (0.22-0.57) | 0.20 (0.15-0.27) | 8 hr. | — | — | — | 7 | 9 |
| Propylamine | 0.57* | 0.56 (0.30-1.02) | 2 min.§ | 8,000 | 4 | 5/6 | 6 | 9 |
| N,N,N',N'-Tetramethyl-dipropylenetriamine | 1.62 (1.02-2.60) | 0.31 (0.06-1.50) | 8 hr. | — | — | — | 6 | 9 |
| Triallylamine | 1.03 (0.83-1.28)* | 0.40 (0.29-0.54) | 15 min. | 500† | 4 | 1/6 | 5 | 2 |
| Trisooctylamine | 1.62 (1.24-2.13) | 3.18 (2.35-4.29) | — | — | — | — | 7 | 1 |
| **Aromatic Nitro and Amino Compounds** | | | | | | | | |
| p-Chloroaniline | 0.31 (0.20-0.48)* | 0.36* | 4 hr. | — | — | — | — | 8 |
| a-Chloro-p-nitrostyrene | 0.71* | 0.39 (0.29-0.53)* | — | — | — | — | 6 | 7 |
| N,N-Dimethylaniline | 1.41 | 1.77 (1.09-2.86) | 8 hr. | — | — | — | 3 | 5 |
| 2,4-Dinitrochlorobenzene | 1.07 (0.77-1.50)* | 0.13 (0.08-0.19)* | — | — | — | — | 7 | 10 |
| N-(2-Ethylhexyl)aniline | 2.41 (1.88-3.23)* | 7.1 (2.7-18.5) | 8 hr. | — | — | — | 5 | 1 |
| 2-Methyl-1 -naphthylamine | 0.62 (0.47-0.81)* | 2.83 | — | — | — | — | 2 | 2 |
| 1-(p-Nitrophenyl) ethyl nitrate | 2.14 (1.54-2.99)*, ** | 14.1 (8.8-22.9) | 8 hr. | — | — | — | 2 | 1 |
| o-Toluidine | 0.94 (0.67-1.31)* | 3.25 (2.01-5.25) | 8 hr. | — | — | — | 2 | 8 |
| **Amide and Imide Compounds** | | | | | | | | |
| N,N-Diethylacetoaceta-mide | 4.76 (3.39-6.68) | — | 8 hr. | — | — | — | 1 | |
| N,N-Dimethylacetamide | 5.63* | 2.24 (1.23-4.11) | 8 hr. | — | — | — | 2 | 8 |
| N,N-Dimethylaceto-acetamide | 22.6 | 14.1 | 8 hr. | — | — | — | 2 | 2 |
| 2,3-Dimethyl carbamic acid, m-isopropyl phenyl ester | 0.16 (0.12-0.21)* | 0.28 (0.18-0.46) | — | — | — | — | 2 | 2 |
| 2,3-Dimethylphenyl maleimide | 0.71 (0.44-1.14)* | — | 8 hr. | — | — | — | 7 | 8 |
| N-Ethylacetoacetamide | 14.2 (8.9-22.5) | — | 8 hr. | — | — | — | 1 | 5 |
| N-(2-Ethylbutoxyethoxy-propyl)bicyclo[2.2.1] heptene-2,3-dicarboximide | 9.5 (6.7-13.4)* | 16.0 (10.8-23.6) | — | — | — | — | 5 | 5 |

RANGE-FINDING TOXICITY DATA—CONTINUED

| Material Studied | Single Oral $LD_{50}$ for Rats Ml/Kg | Single Skin Penetration $LD_{50}$ for Rabbits Ml/Kg | Concentrated Vapor Inhalation by Rats Maximum for No Death | Inhalation of Metered Vapor Concentration by Rats: Concentration ppm | Time, hr. | Mortality | Irritation on Uncovered Rabbit Belly | Corneal Injury in Rabbits |
|---|---|---|---|---|---|---|---|---|
| N-Methyl-N-vinylacetamide | 2.83 | 1.41 | 4 hr. | 4,000 | 4 | 0/5 | 1 | 7 |
| p-Tolylmaleimide | 0.71 (0.48-1.05)* | 0.56 (0.22-0.58)* | 8 hr. | — | — | — | — | 0 |
| **Heterocyclic Nitrogen Compounds** | | | | | | | | |
| Acrylic acid, 2-(5'ethyl-pyrid-2'-yl)ethyl ester | 4.92 (3.75-6.46) | 2.23 (1.38-3.59) | 8 hr. | — | — | — | 5 | 2 |
| 1-(2-aminoethyl) piperazine | 2.14 (1.54-2.99) | 6.88 (0.34-2.31) | 8 hr. | — | — | — | 6 | |
| 1,4-Di-(2-hydroxyethyl) piperazine | 3.73 (2.68-5.21) | >10 | — | — | — | — | 3 | 5 |
| 1-[2-(N,N-Dimethylamino) ethyl]-4-methyl-piperazine | 1.42 (0.96-2.08) | 0.39 (0.29-0.53) | 8 hr. | — | — | — | 6 | 8 |
| 2,6-Dimethylmorpholine | 2.83* | 0.71 (0.39-1.30) | 4 hr. | 4,000 | 4 | 0/6 | 2 | 7 |
| 5-Ethyl-2-methylpyridine-1-oxide | 2.00 (1.28-3.13) | 1.77 | — | — | — | — | 2 | 5 |
| Hexahydro-1,4-diazepine | 2.83* | 1.05 (0.75-1.48) | 8 hr. | — | — | — | 6 | 9 |
| 2-Hydroxyethyl-5-ethyl-pyridine | 4.29 (3.07-5.98)* | 1.78 (0.97-3.26) | — | — | — | — | 2 | 7 |
| 1-(2-Hydroxyethyl) piperazine | 4.92 (3.75-6.46) | >5.0 | 8 hr. | — | — | — | 1 | 5 |
| Indole | 1.00 (0.64-1.57)* | 0.79 (0.59-1.07)* | 8 hr. | — | — | — | — | 8 |
| 1-Methylpiperazine | 2.83 | 1.49 (0.88-2.50) | 8 hr. | — | — | — | 6 | 8 |
| 1-Phenylpiperazine | 0.21 (0.13-0.34)* | 0.14 (0.08-0.25) | 8 hr. | — | — | — | 6 | 9 |
| Piperidine | 0.52 (0.42-0.64)* | 0.32 (0.23-0.43) | 15 min.§ | 2,000† | 4 | 0/6 | 6 | 9 |
| 1-(5,5,7,7-Tetramethyl-2-octanyl)-2-methyl-5-ethylpyridinium chloride | 0.54 (0.38-0.75)* | 0.071 (0.044-0.11)* | — | — | — | — | 7 | 9 |
| **Nitriles** | | | | | | | | |
| Acetic acid, 1-cyanovinyl ester | 0.10 (0.08-0.14)* | 0.14 (0.08-0.26) | 5 min. | 125 | 4 | 4/6 | 4 | 10 |
| 2-Acetoxyisosuccinodinitrile | 0.12 (0.10-0.15)* | 0.11 (0.06-0.20)* | 1 hr. | — | — | — | — | 9 |
| Acrylic acid, 2-(2-cyanoethoxy)ethyl ester | 1.12 (0.84-1.50) | 0.75 (0.51-1.11) | 8 hr, | — | — | — | 2 | 5 |
| Acrylic acid, 2-cyanoethyl ester | 0.18 (0.13-0.24)* | 0.22 | 8 hr. | — | — | — | 5 | 8 |
| Allyl cyanide | 0.12 (0.10-0.14)* | 1.41 | 5 min. | 250† | 4 | 0/6 | 2 | 3 |
| Butyronitrile | 0.14 (0.10-0.19)* | 0.50 (0.37-0.68) | — | 1,000 | 4 | 5/6 | 1 | 2 |
| Chloroacetonitrile | 0.22 (0.17-0.29)* | 0.071 | 15 min.§ | 250† | 4 | 1/6 | 2 | 5 |
| 4-Cyanoethoxy-2-methyl-2-pentanol | 3.2* | 1.5 | — | — | — | — | 2 | 5 |
| 6-Cyanohexanoic acid, ethyl ester | 11.2 | 7.07 | 8 hr. | — | — | — | 3 | 2 |
| N-Cyanomethylmorpholine, 50% | 1.23 (0.94-1.61) | 0.20 (0.15-0.27) | 4 hr. | — | — | — | 1 | 5 |
| 3-Cyanopropionic acid, ethyl ester | 10.3 (8.3-12.8) | — | 4 hr. | — | — | — | 1 | 2 |
| Diethylene glycol mono-2-cyanoethyl ether | 13.4 (8.2-21.8)*, ** | — | 8 hr. | — | — | — | 2 | 1 |
| 2,4-Dihydroxy-3,3-dimethylbutyronitrile | 0.31 (0.24-0.41)* | 0.13 (0.09-0.17) | 8 hr. | — | — | — | 1 | 7 |

RANGE-FINDING TOXICITY DATA—CONTINUED

| Material Studied | Single Oral $LD_{50}$ for Rats Ml/Kg | Single Skin Penetration $LD_{50}$ for Rabbits Ml/Kg | Concentrated Vapor Inhalation by Rats Maximum for No Death | Inhalation of Metered Vapor Concentration by Rats | | | Irritation on Uncovered Rabbit Belly | Corneal Injury in Rabbits |
|---|---|---|---|---|---|---|---|---|
| | | | | Concentration ppm | Time, hr. | Mortality | | |
| Dimethylamino acetonitrile | 0.050 (0.032-0.079)* | 0.17 (0.12-0.24) | 10 min. | 125† | 4 | 0/6 | 2 | 5 |
| 3-(Dimethylamino) propionitrile | 2.60 (1.98-3.41) | 1.41 (0.83-2.29) | 8 hr. | — | — | — | 1 | 5 |
| Glycolonitrile, 70% | 0.016 (0.013-0.020)* | 0.0050 (0.0034-0.0074)* | 8 hr. | 250 | 4 | 4/6 | 2 | ‡ |
| 4-Hydroxyisobutyronitrile | 0.017 (0.011-0.026)* | 0.017 (0.012-0.024) | 5 min. | 62.5† | 4 | 2/6 | 1 | ‡ |
| Isobutyronitrile | 0.10 (0.077-0.13)* | 0.31 (0.21-0.46) | 10 min.§ | 500† | 4 | 0/6 | 1 | ‡ |
| Methacrylonitrile | 0.25 (0.19-0.32) | 0.35 (0.19-0.67) | 15 min.§ | 1,000† | 4 | 4/6 | 2 | 1 |
| 3-Methyl-3-butenonitrile | 0.54 (0.39-0.75)* | 2.83 | 15 min. | 1,000† | 4 | 1/6 | 1 | 2 |
| Trichloroacetonitrile | 0.25 (0.19-0.32)* | 0.99 (0.49-1.64) | 5 min.§ | 125† | 4 | 0/6 | 6 | 10 |
| **Halogen Compounds** | | | | | | | | |
| 3-Chloro-2-fluoro-1-propene | 0.28 (0.21-0.37) | 0.20 (0.09-0.44) | — | 1,000 | 4 | 5/6 | 1 | ‡ |
| Chloroform | 2.12 (1.14-4.22)*, ** | — | 5 min.§ | 8,000 | 4 | 5/6 | 2 | — |
| 6-Chlorohexanoic acid | 3.08 (2.49-3.81)* | — | 8 hr. | — | — | — | — | 9 |
| 6-Chlorohexanoic acid, ethyl ester | 4.92 (3.75-6.46) | 7.07 | 8 hr. | — | — | — | 1 | 1 |
| 2-(4-Chlorophenoxy) ethanol | 2.83* | 0.50 (0.37-0.68) | 4 hr. | — | — | — | 2 | 8 |
| Decyl chloride (mixed primary isomers) | 45.3 (14.0-146.8) | 5.66 (3.25-9.85) | 4 hr. | — | — | — | 4 | 1 |
| 1,1-Diacetoxy-2,3-dichloropropane | 0.32* | 1.0 | — | — | — | — | 5 | 10 |
| 2-(1,2 Dichloroethyl)-4-methyl-1,3-dioxolane | 0.62 (0.47-0.81)* | 1.01 (0.74-1.36) | — | — | — | — | 2 | 2 |
| 2,3-Dichloro-2-methyl-propionaldehyde | 1.62 (1.24-2.13)* | 0.36 (0.19-0.68) | 15 min. | 250† | 4 | 1/6 | 2 | 8 |
| 1,3-Dichloro-2-propanol | 0.11 (0.059-0.20)* | 0.80 (0.54-1.18) | 30 min. | 125† | 4 | 2/6 | 2 | 8 |
| 2,3-Dichloro-1-propene | 0.32 (0.26-0.40)* | 1.58 (1.16-2.13) | 15 min.§ | 500† | 4 | 3/6 | 5 | 5 |
| 2,3-Dichloropropionic acid | 0.42 (0.25-0.69)* | 0.40 (0.18-0.88)* | — | — | — | — | 7 | 9 |
| 2-Fluoro-2-propene-1-ol | 0.13 (0.071-0.24)* | 0.0031 (0.0014-0.0068) | — | 1,000 | 1 | 4/6 | 1 | ‡ |
| 1,1,3 Tri (2-chloroethoxy)propane | 0.71* | 6.3 | 1 hr. | — | — | — | 1 | 1 |
| 1,2,2-Trichloro-1,1,3,3,3-pentafluoropropane | 14.9 (10.7-20.8) | >20 | — | 8,000† | 4 | 1/6 | 1 | 3 |
| 1,1,3-Trichloropropane | 7.46 (5.35-10.4)* | >20 | 30 min.§ | 8,000 | 4 | 4/6 | 4 | 2 |
| 1,2,3 Trichloropropane | 0.33 (0.26-0.40) | 1.77 | — | 1,000 | 4 | 5/6 | 1 | 4 |
| 1,2,3-Trichloropropene | 0.62 (0.47-0.81)* | 0.64 (0.47-0.85) | 30 min.§ | 500 | 4 | 5/6 | 5 | 5 |
| 2,2,3 Trichloropropionaldehyde | 0.24 (0.17-0.33) | 0.71 | 2 hr. | — | — | — | 6 | 8 |
| 2,2,3-Trichloropropionic acid | 2.46 (1.88-3.23)* | 1.77 (1.09-2.86)* | — | — | — | — | 7 | 9 |
| **Sulphur Compounds** | | | | | | | | |
| Acrylic acid, 2-methyl-, dimethyl ester | 1.23 (0.94-1.62) | 1.49 (0.89-2.47) | — | — | — | — | 6 | 4 |
| 2-Acryloxyethyl dimethylsulfonium methyl sulfate | 1.87 (1.34-2.60) | 2.00 (0.51-7.78) | — | — | — | — | 4 | 5 |
| Benzenesulfonic acid | 0.89 (0.36-2.21) | — | 8 hr. | — | — | — | 7 | 9 |

RANGE-FINDING TOXICITY DATA—CONTINUED

| Material Studied | Single Oral $LD_{50}$ for Rats Ml/Kg | Single Skin Penetration $LD_{50}$ for Rabbits Ml/Kg | Concentrated Vapor Inhalation by Rats Maximum for No Death | Inhalation of Metered Vapor Concentration by Rats | | | Irritation on Uncovered Rabbit Belly | Corneal Injury in Rabbits |
|---|---|---|---|---|---|---|---|---|
| | | | | Concentration ppm | Time, hr. | Mortality | | |
| 2-Ethylhexenyl sulfonate, sodium salt | 25.8 (19.7-33.9) | >10 | 8 hr. | — | — | — | 3 | 4 |
| Methyl sulfoxide | 20.5 (16.9-25.3)* | — | 4 hr. | — | — | — | 2 | 2 |
| Methyl vinyl sulfone | 0.57* | 0.032 (0.013-0.079) | 8 hr. | — | — | — | 2 | 5 |
| Phenyl sulfide | 0.49 (0.38-0.65) | 11.3 | 8 hr. | — | — | — | 5 | 2 |
| Sulfamic acid, 2-aminoethyl ester | 7.46 (5.35-10.4)* | 14.1 | 4 hr. | — | — | — | 1 | 1 |
| Vinyl S-(butylmercapto-ethyl) ether | 2.83* | 10 | 8 hr. | — | — | — | 5 | 2 |
| Vinyl S-(ethylmercapto-ethyl) ether | 4.92 (3.75-6.46)* | >10 | 8 hr. | — | — | — | 2 | 2 |
| Vinyl sulfone | 0.032 (0.026-0.043)* | 0.022 (0.014-0.036) | 8 hr. | — | — | — | 7 | 5 |
| **Phosphorous Compounds** | | | | | | | | |
| P-Chloro-5,10-dimethyl-2,4-dioxa-P-thiono-3-phosphabicyclo[4.4.0,decane | 0.11 (0.071-0.18) | 0.20 (0.09-0.44) | — | — | — | — | 2 | 2 |
| 2-Chloro-4,6-dimethyl-2-oxo-1,3,2-dioxaphosphorinane | 0.38 (0.18-0.61)* | 0.50 | — | — | — | — | 4 | 9 |
| P-Chloro-2,4-dioxa-5-ethyl-P-thiono-3-phosphabicyclo[4.4.0]decane | 0.14* | 0.40 (0.27-0.59) | — | — | — | — | 4 | 1 |
| P-Chloro-2,4-dioxa-5-methyl-P-thiono-3-phosphabicyclo[4.4.0,decane | 0.12 (0.086-0.16)* | 0.36 | — | — | — | — | 2 | 1 |
| 2-Chloro-5-ethyl-2-oxo-4-propyl-1,3,2-dioxaphosphorinane | 0.025 (0.020-0.030)* | 1.41 | — | — | — | — | 5 | 9 |
| 2-Chloro-5-ethyl-4-propyl-2-thiono-1,3,2-dioxaphosphorinane | 0.30 (0.18-0.49)* | 1.00 (0.74-1.35) | — | — | — | — | 1 | 1 |
| Chlorophosphoric acid, diethyl ester | 0.011 (0.010-0.012)* | 0.0079 (0.0059-0.011) | — | — | — | — | 2 | 1 |
| 2-(Dibutylphosphono) succinic acid, dibutyl ester | 25.2* | >10 | 8 hr. | — | — | — | 2 | 1 |
| 2-(Dibutylphosphono) succinic acid, di-(2-ethylhexyl)ester | >40* | >20 | 8 hr. | — | — | — | 2 | 1 |
| O,O'-Di(2-ethylhexyl) dithiophosphoric acid | 2.14 (1.54-2.99) | 1.25 (0.50-3.14) | — | — | — | — | 7 | 8 |
| Di-(2-ethylhexyl) phosphonic acid | 4.94 (3.76-6.46) | 1.25 (0.57-2.74) | 8 hr. | — | — | — | 6 | 9 |
| Phosphoric acid, di-(2-ethylhexyl) hydroxypropyl ester | 11.3* | — | 8 hr. | — | — | — | 3 | 4 |
| Phosphorothioic acid, tri(2-chloroethyl) ester | 0.82 (0.62-1.07)* | 1.8 | 8 hr. | — | — | — | 2 | 4 |
| Tributylphosphine sulfide | 0.93 (0.67-1.30) | 1.00 (0.68-1.48) | 8 hr. | — | — | — | 4 | 2 |
| Triisooctyl phosphine | 21.4 (15.4-29.9) | 3.97 (2.93-5.37) | — | — | — | — | 4 | 1 |
| **Silicon Compounds** | | | | | | | | |
| (4-Aminobutyl)diethoxy (methyl)silane | 6.50 (4.06-10.4)* | 0.045 (0.015-0.136) | 8 hr. | — | — | — | 3 | 8 |
| (4-Aminobutyl)triethoxysilane | 1.62 (1.02-2.60) | 2.50 (1.69-3.70) | 8 hr. | — | — | — | 4 | 9 |

RANGE-FINDING TOXICITY DATA—CONCLUDED

| Material Studied | Single Oral $LD_{50}$ for Rats Ml/Kg | Single Skin Penetration $LD_{50}$ for Rabbits Ml/Kg | Concentrated Vapor Inhalation by Rats Maximum for No Death | Inhalation of Metered Vapor Concentration by Rats | | | Irritation on Uncovered Rabbit Belly | Corneal Injury in Rabbits |
|---|---|---|---|---|---|---|---|---|
| | | | | Concentration ppm | Time, hr. | Mortality | | |
| (3-Aminopropyl) diethoxy(methyl) silane | 4.76 (3.35-6.75) | 2.52 (1.86-3.41) | 8 hr. | — | — | — | 3 | 8 |
| (3-Aminopropyl)triethoxysilane | 1.78 (0.93-3.41)* | — | 8 hr. | — | — | — | 6 | 8 |
| Amyltrichlorosilane | 2.34 (1.98-2.77)* | 0.78 (0.59-1.02) | 4 hr. | 1,000† | 8 | 0/6 | 6 | 9 |
| (2-Carbethoxyethyl) diethoxy(methyl) silane | 20.8 (14.3-30.3) | >10 | 8 hr. | — | — | — | 2 | 1 |
| (2-Carbethoxyethyl) triethoxysilane | 22.4 (16.2-30.8) | — | 8 hr. | — | — | — | 2 | 1 |
| (2-Carbethoxypropyl) diethoxy(methyl) silane | 24.6 (18.8-32.3) | — | 8 hr. | — | — | — | 8 | 5 |
| (3-Carbethoxypropyl) diethoxy(methyl) silane | 22.4 | — | 8 hr. | — | — | — | 1 | 5 |
| (2-Cyanoethyl) trichlorosilane | 2.00 (1.28-3.13)* | — | 8 hr. | — | — | — | 6 | 10 |
| (2-Cyanoethyl) triethoxysilane | 5.63* | 5.95 (3.57-9.90) | 4 hr. | — | — | — | 2 | 1 |
| (3-Cyanopropyl) dichloro(methyl) silane | 2.83* | 1.49 (0.67-3.29) | 8 hr. | — | — | — | 6 | 8 |
| (3-Cyanopropyl) diethoxy(methyl) silane | 3.73 (2.68-5.21)* | — | 8 hr. | — | — | — | 2 | 1 |
| (3-Cyanopropyl) methylsiloxane, cyclic tetramer | >64 | — | 8 hr. | — | — | — | 1 | 1 |
| (3-Cyanopropyl) trichlorosilane | 2.83* | — | 4 hr. | — | — | — | 6 | 9 |
| (3-Cyanopropyl) triethoxysilane | 4.92 (3.75-6.46)* | >10 | 8 hr. | — | — | — | 2 | 1 |
| (3,4-Cyclohexenyl) trichlorosilane | 2.83* | 0.63 (0.39-1.02) | 8 hr. | — | — | — | 6 | 9 |
| Di-(2-ethylhexoxy)-di-(2-ethylbutoxy)silane | 56.3 (35.5-89.4) | >20 | 8 hr. | — | — | — | 1 | 1 |
| Ethenylvinyl) dichlorosilane | 2.83* | 0.75 (0.54-1.06) | — | 4,000† | 4 | 0/6 | 6 | 9 |
| (2-Phenylethyl) trichlorosilane | 2.83* | 0.74 (0.45-1.24) | 8 hr. | — | — | — | 6 | 8 |
| (Phenyl)trifluorosilane | 0.31 (0.24-0.40)* | 0.64 (0.47-0.86) | 15 min.§ | 1,000† | 4 | 1/6 | 5 | 10 |
| Tetra-(2-ethylhexoxy) silane | >64 | >20 | 8 hr. | — | — | — | 2 | 1 |

* gm/kg in a suitable vehicle
† male rats
‡ inhalation time shown killed all six rats
§ vapor concentration shown killed all six rats
‖ caused death when introduced into the rabbit eye

Stecher, P. G., Ed. 1968

Tall Oil
The Merck Index - An Encyclopedia of Chemicals and Drugs, 8th Edition

pages 1; 621; 763-764; 951; 982

U.S. Department of Agriculture

Fats, oils, tall oil, glycercine:
Wholesale prices per pound, 1967-1971

Agricultural Statistics, U.S. Dept.
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U.S. Department of Commerce, 1970, 1972

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U.S. Foreign Trade Imports Commodity by Country, U.S. Department of Commerce, Washington, D.C.

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U.S. Public Health Service, 1961-67

Oleic acid. Survey of Compounds Which Have Been Tested for Carcinogenic Activity.

U.S. Public Health Service, Department of Health, Education and Welfare

PHS Publication 149, Vol. 1961-1967
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